PARASITOLOGY

LONDON
CAMBRIDGE UNIVERSITY PRESS
FETTER LANE, E.C. 4

CHICAGO: THE UNIVERSITY OF CHICAGO PRESS
(Agents for the United States)
BOMBAY, CALCUTTA, MADRAS: MACMILLAN AND CO., LTD.
TOKYO: MARUZEN COMPANY, LTD.

All rights reserved

PARASITOLOGY

EDITED BY

GEORGE H. F. NUTTALL, F.R.S.

QUICK PROFESSOR OF BIOLOGY AND DIRECTOR OF THE MOLTENO INSTITUTE FOR RESEARCH IN PARASITOLOGY IN THE UNIVERSITY OF CAMBRIDGE

ASSISTED BY

D. KEILIN, F.R.S.

AND

E. HINDLE, PH.D., SC.D.

VOLUME XXV 1933

CAMBRIDGE

AT THE UNIVERSITY PRESS

1933

PRINTED IN GREAT BRITAIN

Medical
Taxon

CONTENTS

No. 1 (FEBRUARY)

PAGE

COWDRY, E. V. and DANKS, W. B. C. Studies on East Coast Fever. II. Behaviour of the Parasite and the Development of Distinctive Lesions in susceptible Animals. (With Plates I–X, containing Figs. 1–97, 2 Diagrams and 4 Charts in the Text) . 1

WORMALL, A. Some Observations on the Value and the Mechanism of the Red-Cell Adhesion Test for Trypanosomiasis of Man and other Animals 64

STRICKLAND, C. and ROY, D. N. The Prophylaxis of Malaria by Drugs 77

JAMES, S. P., NICOL, W. D. and SHUTE, P. G. *Plasmodium ovale* Stephens 1922. (With Plate XI, containing Figs. 1–14) . . 87

TATE, P. and VINCENT, M. The Action of Plasmoquine on Mosquito-induced Malaria of Birds 96

KOTLÁN, A. and POSPESCH, L. Coccidial Infections in the Badger *Meles taxus* L. (With Plate XII, containing Figs. 1–16) . . 102

LEIGH-SHARPE, W. HAROLD. Note on the Occurrence of *Graffilla gemelipara* Linton (Turbellaria) at Plymouth. (With 1 Figure in the Text) 108

LEIGH-SHARPE, W. HAROLD. A List of British Fishes with their Characteristic Parasitic Copepoda 109

LEIGH-SHARPE, W. HAROLD. A Second List of Parasitic Copepoda of Plymouth with a Description of Three New Species. (With 5 Figures in the Text) 113

MARSHALL, J. F. and STALEY, J. *Theobaldia* (*Culicella*) *litorea* (Shute) n.sp. (Diptera, Culicidae). (With 2 Figures in the Text) 119

FERRIS, G. F. A New Species of *Polyplax* (Anoplura). (With 2 Figures in the Text) 127

HARWOOD, PAUL D. The Helminths Parasitic in a Water Moccasin (Snake) with a Discussion of the Characters of the Proteocephalidae. (With 1 Figure in the Text) 130

No. 2 (April)

PAGE

Mackinnon, D. L. and Ray, H. N. The Life-Cycle of two Species of "*Selenidium*" from the Polychaete Worm *Potamilla reniformis*. (With Plates XIII–XVII, containing Figs. 1–38, and Figs. I–XVIII in the Text) 143

Bishop, Ann. The Morphology and Division of *Hexamita gigas* n.sp. (Flagellata). (With Plate XVIII, containing Figs. 1–13, and 10 Figures in the Text) 163

Duke, H. Lyndhurst. The Domestic Fowl of Uganda as a Host for Trypanosomes of the *Brucei* Group 171

Li, Hsi-Chieh. Report on a Collection of Parasitic Nematodes, mainly from North China. Part I. Filarioidea. (With 53 Figures in the Text) 192

Daubney, R. Trichostrongylid Nematodes from Sheep in Kenya. (With 17 Figures in the Text) 224

Willey, Charles H. The Lymph System of *Zygocotyle lunatum* (Trematoda, par Amphistomidae). (With 3 Figures in the Text) 242

Jones, E. Idris. On *Ceratotrema furcolabiata* n.g. et n.sp. and *Hemipera sharpei* n.sp., Two New Digenetic Trematodes of British Marine Fishes. (With 9 Figures in the Text) . . 248

Leigh-Sharpe, W. Harold. The Hirudinea of Plymouth. Part I. (With 6 Figures in the Text) 255

Varley, G. C. and Butler, C. G. The Acceleration of Development of Insects by Parasitism 263

Lewis, E. Aneurin. *Rhipicephalus ayrei* n.sp. (a Tick) from Kenya Colony. (With 2 Figures in the Text) 269

Macleod, J. and Gordon, W. S. Studies in Tick-borne Fever of Sheep. I. Transmission by the Tick, *Ixodes ricinus*, with a Description of the Disease Produced. (With 4 Temperature Charts) 273

Corrigenda 284

No. 3 (July)

Keilin, D. and Robinson, V. C. On the Morphology and Life History of *Aproctonema entomophagum* Keilin, a Nematode Parasite in the Larvae of *Sciara pullula* Winn. (Diptera—Nematocera). (With Plates XIX and XX, containing Figs. 1–19, and Figs. I, II in the Text) 285

PAGE

OTTER, G. W. On the Biology and Life History of *Rhabditis pellio* (Nematoda). (With 1 Graph) 296

BAYLIS, H. A. A New Species of the Nematode Genus *Uncinaria* from a Sea-lion, with some Observations on Related Species. (With 9 Figures in the Text) 308

BROWN, F. J. On the Excretory System and Life History of *Lecithodendrium chilostomum* (Mehl.) and other Bat Trematodes, with a Note on the Life History of *Dicrocoelium dendriticum* (Rudolphi). (With Plate XXI, containing Figs. 1–6, and Fig. I in the Text) 317

JONES, E. IDRIS. Studies on the Monogenea (Trematoda) of Plymouth. I. *Microbothrium caniculae* (Johnstone 1911). (With 3 Figures in the Text) 329

MOGHE, M. A. Four New Species of Avian Cestodes from India. (With 14 Figures in the Text) 333

FULLER, MARY E. The Life History of *Onesia accepta* Malloch (Diptera, Calliphoridae). (With Plate XXII, containing Figs. 1–5, and Figs. I–X in the Text) 342

DUNN, LAWRENCE H. Two New Species of Ticks from Panama (*Amblyomma tapirellum* and *A. pecarium*). (With 4 Figures in the Text) 353

HOARE, CECIL A. Studies on some New Ophidian and Avian Coccidia from Uganda, with a Revision of the Classification of the Eimeriidea. (With 6 Figures in the Text) 359

YAKIMOFF, W. L., MATIKASCHWILI, I. L., RASTEGAÏEFF, E. F. and LEWKOWITSCH, E. N. Coccidia of the Felidae. (With 13 Figures in the Text) 389

RAY, HARENDRANATH. On the Gregarine, *Lankesteria culicis* (Ross), in the Mosquito, *Aedes* (*Stegomyia*) *albopictus* Skuse. (With Plate XXIII) 392

BECKER, E. R. and HALL, PHOEBE R. The Possible Rôle of Inheritance in the Quantitative Character of a Coccidian Infection of the Rat 397

REED, NANCY. Sporogony in *Selenidium mesnili* Brasil, a Sporozoan Parasite of *Myxicola infundibulum* Mont. (With Plates XXIV–XXVII, containing Figs. 1–32, and Figs. I–III in the Text) . 402

No. 4 (December)

PAGE

Tate, P. and Vincent, M. The Action of Synthetic Quinoline Compounds on Avian Malaria 411

Gibbins, E. G. Morphological Study of Malaria Pigment in Oocysts of Naturally Infected *Anopheles*. (With 4 Figures in the Text) 428

Dobell, Clifford. Researches on the Intestinal Protozoa of Monkeys and Man. V. The *Endolimax* of Macaques . . 436

Ferris, G. F. The Mallophagan Genus *Trichophilopterus*. (With 2 Figures in the Text) 468

Roubaud, E. et Colas-Belcour, J. *Argas boueti*, Nouvelle Espèce d'Ixodidé parasite des Chauve-Souris au Soudan Français. (Avec Planche XXVIII, Figs. 1–8, et Figs. I–VI dans le texte) 472

Olenev, N. O. and Rozhdestvenskaja, V. S. A Pathological Condition observed in Ticks (Ixodidae). With Plate XXIX, containing Figs. 1 and 2, and Figs. I–III in the Text) . . . 478

Leigh-Sharpe, W. Harold. *Acespadia pomposa* n.g. et sp. A Parasitic Copepod of *Rhyncodon typicus*. (With 2 Figures in the Text) 480

Harper, W. F. A Cysticercoid from *Helodrilus* (*Allolobophora longus* Cede) and *Lumbricus terrestris* L. (With 1 Figure in the Text) . 483

Woodland, W. N. F. On two new Cestodes from the Amazon Siluroid Fish *Brachyplatystoma vaillanti* Cuv. and Val. (With Plate XXX, containing Figs. 1–22) 485

Woodland, W. N. F. On a new Subfamily of Proteocephalid Cestodes—the Othinoscolecinae—from the Amazon Siluroid Fish *Platystomatichthys sturio* (Kner). (With Plate XXXI, containing Figs. 1–20, and Fig. I in the Text) 491

Canavan, William P. N. A Redescription of *Distomum incommodum* Leidy, from *Alligator mississippiensis*, and Creation of a new Genus (*Homoscaphis*) for it. (With 2 Figures in the Text) 501

Hunter, III, George W. The Strigeid Trematode, *Crassiphiala ambloplitis* (Hughes 1927). (With 6 Figures in the Text) . 510

Talbot, S. Benton. Life History Studies on Trematodes of the Subfamily Reniferinae. (With 24 Figures in the Text) . . 518

Index to Authors 547

Index to Subjects 550

VOLUME XXV FEBRUARY, 1933 No. 1

STUDIES ON EAST COAST FEVER. II. BEHAVIOUR OF THE PARASITE AND THE DEVELOPMENT OF DISTINCTIVE LESIONS IN SUSCEPTIBLE ANIMALS[1].

BY E. V. COWDRY AND W. B. C. DANKS.

Anatomical Laboratory, Washington University, Saint Louis.

(With Plates I–X, containing Figs. 1–97, 2 Diagrams and 4 Charts in the Text.)

CONTENTS.

	PAGE
I. Introduction	2
II. Material	3
III. Technique	8
IV. Observations	9
(1) Parasites in the feeding ticks	9
(2) Changes in the skin caused by the ticks	11
(3) Failure to find parasites at the site of feeding	13
(4) Migration of parasites to lymph glands	14
(5) Kinds of cells parasitised	15
(6) Appearance of agamonts	16
(7) Appearance of gamonts	18
(8) Lesions in lymph glands	19
(9) Blood forming organs	25
(10) Vascular system	26
(11) Endocrines	28
(12) Alimentary tract and pancreas	29
(13) Urino-genital system	29
(14) Respiratory system	31
(15) Nervous system	31
(16) Blood stream	31
V. Discussion	38
(1) Life cycle of *Theileria parva* in cattle	38
(2) Reaction of the tissues to the parasites	42
(3) Mechanism of recovery	46
(4) Prevention	47
(5) Therapy	49
(6) Systematic position of *T. parva*	52
VI. Summary	54
References	56
Explanation of Plates	59

[1] Aided by a grant made by the Rockefeller Foundation to Washington University for Research in Science.

I. Introduction.

Part I, "The life cycle of the parasite in ticks" (Cowdry and Ham, 1930 and 1932[1]), dealt with studies on the behaviour of the parasite, *Theileria parva* in four series of ticks (*Rhipicephalus appendiculatus*). The first series was fed, as larvae, on blood containing parasites; and the remaining three were fed as nymphs, likewise on the blood of animals during typical attacks of East Coast fever.

It was found that parasites leave the ingested red blood cells and come to occupy a position in the lumen of the gut over a relatively long period from shortly after the time of engorgement to as late as 6 days. We were not successful in distinguishing between male and female forms in the lumen of the gut, or in observing the process of fertilisation, though it probably takes place in this location. Many parasites are destroyed in the lumen and in pedunculated epithelial cells. Some, however, continue to develop as zygotes in non-pedunculated cells lining the gut, where they increase greatly in size and, at about the time of moulting, give rise to motile oökinetes. These oökinetes leave the epithelial cells, migrate through the body cavity, and enter the cells of the salivary glands where they change into sporonts, at the periphery of which develop sporoblasts. It is only after the ticks have fed for about 3 days on the animal next in series that the sporoblasts in turn transform into sporozoites which constitute the final infective stages.

It was to determine this life cycle in the tick that we (Cowdry and Ham) accepted the invitation of Lord Passfield, at that time Colonial Secretary, to visit Kenya, as related in Part I, p. 4. Though the question of the possible morphological differentiation of male and female forms and the process of fertilisation remained unclarified, the Director of Agriculture expressed the wish that we should proceed further and investigate the behaviour of the parasites introduced into susceptible animals by the bites of infested ticks and the reactions of the tissues leading to the development of distinctive lesions. We gladly embraced this opportunity, because it is only by learning how the host responds to the parasite and in some cases survives the attack that we can hope rationally to plan therapeutic measures designed to increase its resistance, and others to kill the parasites without at the same time seriously injuring the host. Accordingly, before leaving Kenya, many tissues were collected for detailed examination in the United States. In this second study Mr W. B. C. Danks has taken the place of Dr Arthur W. Ham as junior author. Mr Danks has been enabled to do so by leave granted by the Government of Kenya and by his appointment to a Rockefeller Foundation Fellowship.

The literature has been admirably reviewed by Schilling and Meyer (1930). As in the case of our first contribution our thanks are due to the late Major Eustace Montgomery, Veterinary Adviser to the Secretary of State; the Hon. Alexander Holm, Director of Agriculture, and Major Brassey-Edwards, Chief

[1] See *Parasitology*, **24**, 1.

Veterinary Officer. We are particularly indebted to Mr James Walker, Chief of the Veterinary Research Laboratory, Kabete, for help in many ways and for sending us much material in addition to what we collected in Kenya, without which our investigations would have been greatly handicapped.

II. MATERIAL.

Continuity with Part I of this paper was afforded by the collection of material from the four series of infective ticks and the calves which they fed upon as follows:

(1) *The ticks and the skin to which they were attached.* With a sharp razor wedge-shaped pieces of skin, including some subcutaneous tissue and the attached ticks, were removed. The ticks were separated from the skin by cutting through their probosces with scissors. Numbers were given which made it possible at any time to determine which tick had been feeding on any given piece of skin. The chitin was dissected off and both ticks and skin were fixed separately in Bouin's fluid.

(2) *Excised pieces of lymph glands.* Under chloroform anaesthesia, small pieces of superficial lymph glands were removed with aseptic precautions and fixed in Bouin's fluid.

(3) *Smears from lymph glands and blood.* The gland smears were made with the fluids which entered hypodermic needles plunged through the skin into the superficial lymph glands; and the blood smears with blood taken from the tip of the tail or the margin of one of the ears. The results are listed in Table I.

(4) *Pieces of the principal organs.* These were collected from the calves which died or were killed 21, 8, 14 and 17 days[1] respectively, after infestation with ticks.

Calf No. 5840 (Table I)* showed typical Koch bodies in both gland and blood smears beginning on the 13th day after infestation with the ticks (see Chart I, p. 4).

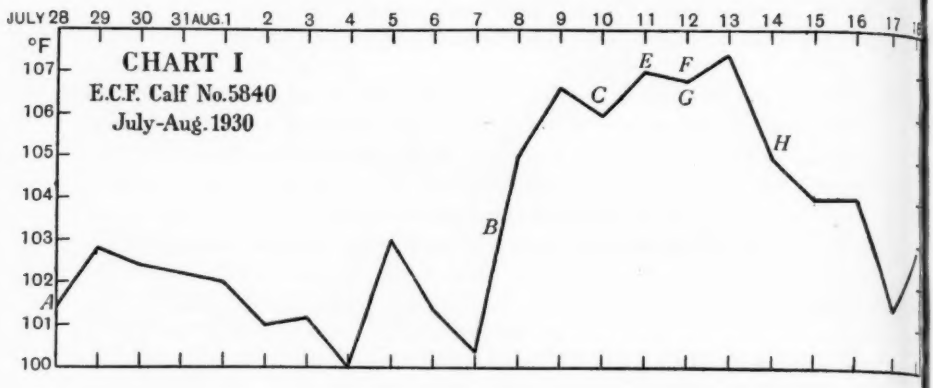
Calf No. 5199 (Table II)* showed parasites in red blood cells 2, 4, 5, 6 and 7 days after infestation with ticks. These parasites were determined as *Theileria mutans*. It appeared altogether too promptly to have been transmitted by our standard series of ticks. On the 8th day this animal died while we were attempting, under chloroform anaesthesia, to remove a piece of a lymph gland for histological study. No Koch bodies were observed in either blood or gland smears, but from our findings with the other three calves it seems likely that the time for their development may not have been sufficient.

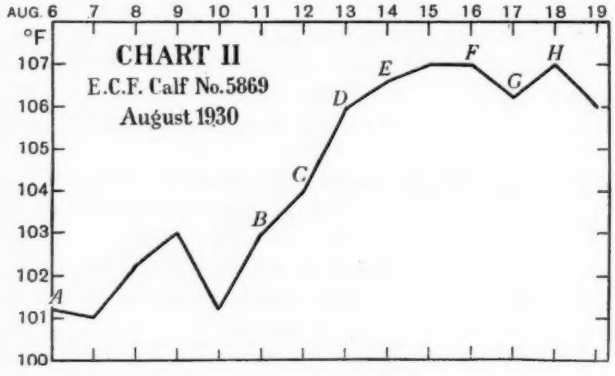
Calf No. 5869 (Table III)* showed Koch bodies in gland smears beginning 8 days after infestation with ticks and in blood smears 4 days later. It developed a characteristic attack of East Coast fever (see Chart II, p. 4).

Calf No. 5838 (Table IV)* acquired the disease more slowly. Koch bodies

[1] These times were erroneously listed in the first paper as 20, 8, 15 and 16 days.

* The asterisk denotes a table published in Part I (Cowdry and Ham, 1932).



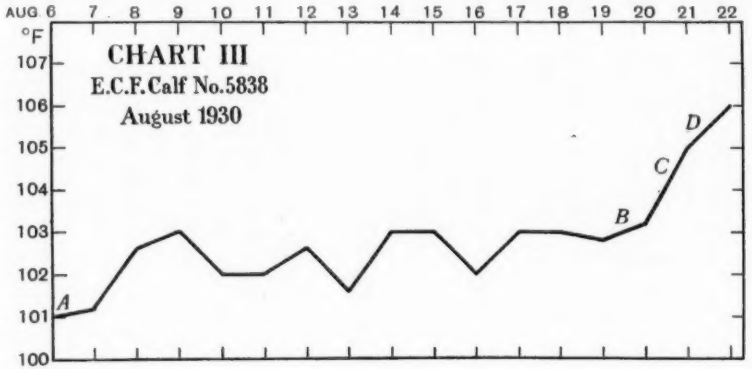


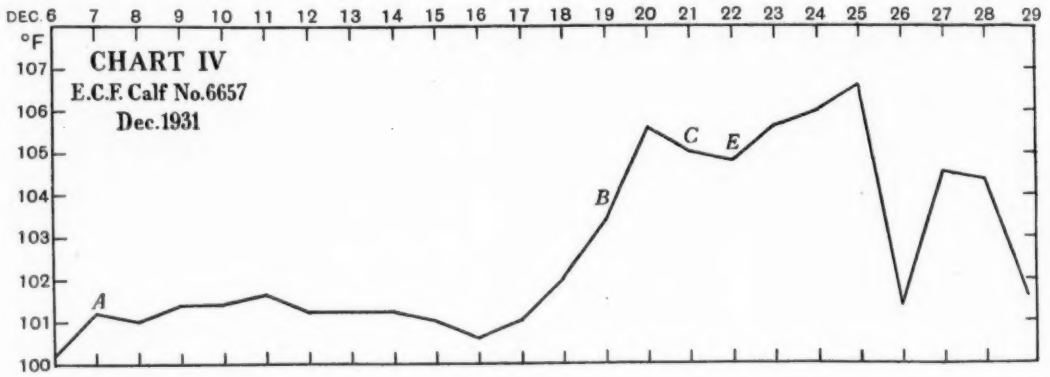
Explanation of Charts I–IV

The time at which various changes occurred are indicated as follows:

A infested with ticks.
B temperature exceeded 103° F.
C agamonts appeared in lymphocytes in gland smears.
D reduction in number of small lymphocytes in sections of excised glands.
E agamonts appeared in lymphocytes in blood smears.
F parasites appeared in red blood cells.
G gamonts appeared in lymphocytes in gland smears.
H gamonts appeared in lymphocytes in blood smears.

(For further details see Table II, p. 18.)


AUG. 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
°F
107
106
105
104
103
102
101
100
CHART III
E.C.F. Calf No. 5838
August 1930
A
B
C
D


DEC. 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
°F
107
106
105
104
103
102
101
100
CHART IV
E.C.F. Calf No. 6657
Dec. 1931
A
B
C
E

were observed in gland smears 15 and 16 days after infestation, but they were not seen in the blood smears. Clinically the case showed, like Nos. 5199 and 5869, all the classical symptoms of East Coast fever, but to a slight degree (see Chart III, p. 5).

It is evident, therefore, that three of our four series of ticks which contained parasites in their salivary glands were capable of transmitting East Coast fever to susceptible animals. It is probable that the other series (Table II)*, which possessed parasites in equal abundance, would also have transmitted the disease if the animal infested had not died so soon. The tissues examined, with the findings, are listed in Tables I and III. Tissues of adults were also studied:

Bovine No. 5657. Died of East Coast fever after repeated doses of benzol and 14 days after exposure to natural East Coast fever infection. Smears and tissues were fixed in Bouin and sublimate acetic mixtures.

Bovine No. 5864. Died of East Coast fever the 3rd day after rise in temperature. Smears of blood were made and a portion of a lymph gland was excised before death.

Tissues from the animals already described were collected while we were in Kenya. The additional material sent by Mr Walker was accompanied by temperature charts and autopsy protocols. The pathological anatomical diagnoses were as follows: further information regarding the febrile reaction, the appearance of parasites and the tissues examined is given in Tables I, II, III and VI.

Bovine No. 5922, black and white grade bull; age about 2½ years. When suffering from East Coast fever typical Koch bodies were found in smears 21 days after exposure to infected ticks. The temperature returned to normal during the 5th week. The animal was killed about 1 year and 1 month after the attack. Autopsy showed nothing but slight distoma infection of the liver.

Calf No. 6657, grade ox; age about 2½ years. Hydrothorax, hyperemia and pulmonary oedema, pleuritis. Icterus of the liver, stasis of bile. Gastroenteritis. Tumour of the spleen. Haemorrhages in cortex of kidneys. Epicardial petechiae[1] (see Chart IV, p. 5).

Bovine No. 6673, grade ox; age about 2 years. Opacity of left cornea. Some lymph glands were hyperemic or haemorrhagic, others showed nothing unusual. Haemorrhages in gastric omentum, mesentery and serosa of small intestine. Haemorrhages in the pleura costalis and epicardium; slight ecchymosis of left endocardium. Subcapsular haemorrhages of the spleen. Occasional haemorrhages in the pharyngeal mucous membrane, sides and ventral surface of the tongue. Gross infection of fourth stomach with *Strongylus contortus*. Mucosal haemorrhages in the small and large intestine. Blood coagula in the lumen of the caecum, colon and rectum. Pigmentation of liver. Haemorrhages in the mucous membrane and serosa of the gall bladder. Fatty

[1] Unless stated to the contrary, animals which were about to die from the disease and were actually *in extremis* were killed by bleeding.

degeneration (?) of the kidneys. Haemorrhages on the surface and within the calyx of the kidneys. Haemorrhages in the mucous membrane and serosa of the bladder were rare.

Bovine No. 6927, light red grade ox; age about 4 years. Inoculated intravenously with 20 c.c. of peptonised spleen pulp of East Coast fever animal No. 7194. Twelve days later temperature exceeded 103° F.; it rose on the 15th day to 106·8° F. and fell within 2 days to normal. On the 18th day after injection there were rare Koch bodies in gland smears. The animal was exposed to natural infection on the 23rd day, but remained normal to the 46th day, when it was sacrificed for collection of material. Ecchymosis of left endocardium. Distoma infection of liver. White foci in cortex of right kidney.

Calf No. 7539, age 5½ months. Pure East Coast fever infection caused experimentally by ticks (Reg. No. 558). Ulcerative gastritis and enteritis. Hyperaemia and pulmonary oedema. Infarcts in kidneys. Inflammation and haemorrhages of lymph glands. Ecchymosis of endocardium.

Bovine No. 7600, red grade ox; age about 3½ years. Enlarged prescapular lymph glands; other lymph glands moderately enlarged. Slight ascites and hydropericardium. Parasitic infection of lungs. Stasis of bile in liver. Hyperaemia of mucosa of gall bladder. Slight icterus of renal cortex. Oedema of fourth stomach. Patchy hyperaemia of duodenum, ileum and caecum, and hyperaemia of colon. Mucus and bile deposit in jejunum.

Calf No. 7613, age 4 months. Pure East Coast fever infection following infestation of right ear with tick nymphs (Reg. No. 557). Killed for collection of tissues but not *in extremis*. Haemorrhages in fourth stomach, caecum and rectum. Ileo-caecal valve haemorrhagic. Infarcts in kidney.

Bovine No. 7644, grade ox; age about 2½ years. Keratitis and corneal opacity. Hydrothorax, hyperaemia and pulmonary oedema. Epicardial and endocardial haemorrhages. Infarcts in kidneys. Haemorrhagic and ulcerative gastritis and oedema of stomach folds. Haemorrhages in colon, and hyperaemia of small intestines. Haemorrhages in gall bladder. Some lymph glands haemorrhagic, others moderately enlarged.

Bovine No. 7837, red and white steer; age about 2 years. Typical East Coast fever infection with Koch bodies frequent; but the date of first exposure is uncertain so that it is not included in Table VI. Interval 12 days. Slight hyperaemia of lungs, fibrotic pleuritis. Ascites and oedema of caecum and colon. Pigmentation of liver. Infiltration of fatty capsule of kidney, of middle mediastinum and of connective tissues surrounding root of lung and the gall bladder. Haemorrhages in the calyx major of the kidney and on the surface of bone marrow (femur). Erosions of tongue.

Bovine No. 7843, black and white bull; age about 15 months. Typical East Coast fever infection with Koch bodies frequent; but the date of first exposure is uncertain—not included in Table VI. Infiltration of folds of fourth stomach. Infiltration of walls of caecum and colon. Bile deposit in intestines. Haemorrhagic foci in lungs. White infarcts (?) and rare pin-point haemorrhages

in kidneys. Diarrhoea. Cachexia. Necrotic areas in tongue. Prescapular and precrural lymph glands slightly enlarged. Lymph glands usually pale and juicy, rarely haemorrhagic.

Blood smears were also secured from the following animals during the disease reaction: Nos. 1869, 3510, 3519, 4810, 5759, 5760, 5774, 5790, 5811 and 7613.

III. Technique.

In the study of the parasite in ticks the principal technical problems were to remove satisfactorily the ticks from their chitinous covering, to secure a good fixation and sharp coloration of sections.

The making of blood and gland smears of the animals fed upon and their proper staining by Giemsa's method are simple procedures which do not require comment. But the satisfactory demonstration of all the parasites present in sections of various tissues is more difficult.

Except for the pieces of skin and of lymph glands which were removed at stated intervals during life, all of the tissues were collected at autopsies made immediately the animals were killed at some particular stage of the disease reaction, or died from it. The usual way was to kill the animals by bleeding, but since this results in draining the tissues of blood (with the contained parasites) two were killed by chloroform and the tissues were fixed with as little haemorrhage as possible for comparison. Care was taken not to allow the surface of the tissue to dry before fixation or to handle the parts to be fixed with forceps. Pieces of tissue were cut with a razor into rectangular slices about 3 mm. thick, 8 mm. long and 5 mm. wide. They were fixed for routine purposes in Bouin's fluid (75 c.c. saturated aqueous picric acid, 25 c.c. commercial formalin and 4 c.c. glacial acetic acid). Not more than four pieces were placed in a bottle containing 100 c.c. of fixative. It was found that if more pieces were fixed in one bottle the results were unsatisfactory. Immediately after plunging the tissues into the fixative the fluid was gently agitated at intervals of about 10 min. to avoid their sticking to the bottom of the bottle and to ensure equal penetration from all sides.

After the fixative had acted for 24 hours it was poured off, the tissues being left at the bottom of the bottle. The tissues were not washed in water, but 35, 50 and 70 per cent. alcohols were added successively and poured off at intervals of about 3 hours, after which they were left in 95 per cent. alcohol over night. Early the following morning the 95 per cent. alcohol was replaced with absolute alcohol which was changed after 1 hour and allowed to remain altogether 4 hours. The tissues were treated, still in the original bottles, with half absolute alcohol and xylol for 1 hour, and cleared in two changes of xylol over a period of 2 hours. Finally they were embedded in paraffin melting at 60° C. This last operation required 3 hours more.

Serial sections were cut 4 microns in thickness and were fixed to slides by the albumin-water method. They usually had a marked yellow colour from

the picric acid still remaining in them. They were passed down through toluol and graded alcohols to distilled water. They were then treated with 1 per cent. potassium permanganate and 5 per cent. oxalic acid and coloured with Giemsa's stain as outlined in detail in the first paper. The preparations should not be differentiated too far because the parasites hold the stain less strongly than the tissue cells.

Sometimes the sections were coloured too intensely blue without the desired red contrast, in which event others of the same kind were immersed in permanganate and oxalic acid diluted 10 to 100 times with distilled water, or were coloured with Giemsa's mixture without any preliminary treatment with potassium permanganate and oxalic acid. When, in rare cases, they were still too blue, no permanganate or oxalic was used; but instead they were placed in a 1 per cent. aqueous solution of potassium bichromate for 1 hour and simply rinsed in distilled water before staining.

Despite every precaution some preparations were secured which did not reveal parasites although we had every reason to believe that they were originally present. For this reason negative observations in respect to parasites in refractory tissues meant very little. Reliance was placed in more perfect specimens which showed them with great clearness. In place of Canada balsam, we mounted all of our Giemsa preparations in the cedar oil ordinarily supplied for use with immersion objectives, because of its almost neutral reaction. This prevented fading of the colours. We were careful never to leave the preparations for any length of time in direct sunlight.

Many sections, fixed in this way, were stained with haematoxylin and eosin. The Feulgen reaction, as specified by Cowdry (1928), was applied to sections of tissues in order to reveal thymonucleic acid. By this technique the nuclei of the parasites and of the tissue cells were coloured specifically but not so intensely as by Giemsa's stain.

IV. OBSERVATIONS.

(1) *Parasites in the feeding ticks.*

Ticks and skin were removed daily from the calves. In all, sixty-six ticks and the pieces of skin to which they were attached were studied in serial sections. Twenty-five of the ticks were found to contain parasites in their salivary glands. These have been described in our first paper and are listed here in Table I; but the results of the examination of the skin as far as parasites are concerned are not given in the table because none were found until the disease was well established (Table III).

Table I. *Results of parallel examination of ticks and calves fed upon.*

Days	Calf 5840				Calf 5199				Calf 5869				Calf 5838			
	Tick	Gland smear	Koch bodies in blood	*T. parva* in blood	Tick	Gland smear	Koch bodies in blood	*T. parva* in blood	Tick	Gland smear	Koch bodies in blood	*T. parva* in blood	Tick	Gland smear	Koch bodies in blood	*T. parva* in blood
1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
2	2−	−	−	−	2−	−	−	*P. mutans*	2+ 1−	−	−	−	1+ 1−	−	−	−
3	1+ 1−	−	−	−	1+ 1−	−	−	*P. mutans*	2+	−	−	−	1−	−	−	−
4	1+ 1−	−	−	−	2−	−	−	*P. mutans*	2+	−	−	−	0	−	−	−
5	1+ 1−	−	−	−	2+	−	−	*P. mutans*	2+ 2−	−	−	−	0	−	−	−
6	2−	−	−	−	1+ 1−	−	−	*P. mutans*	1+ 1−	−	−	−	0	−	−	−
7	1+ 1−	−	−	−	1+ 5−	−	−	*P. mutans*	2+ 2−	−	−	−	0	−	−	−
8	2−	−	−	−	Died under chloroform anaesthesia				2−	+	+	−	0	−	−	−
9	1+ 1−	−	−	−					2−	+	+	−	0	−	−	−
10	1+ 1−	−	−	−					0	+	+	−	0	−	−	−
11	2−	−	−	−					0	+	+	+	0	−	−	−
12	2−	−	−	−					0	+	+	+	0	−	−	−
13	1+ 1−	+	−	−					0	+	+	+	0	−	−	−
14	2−	+	+	−					Killed by chloroform to avoid draining blood from tissues				0	−	−	−
15	0	+	+	+									0	+	−	−
16	0	+	+	+									1+	+	−	−
17	0	+	+	+									Killed by chloroform to avoid draining blood from tissues			
18	0	+	+	+												
19	0	+	+	+												
20	0	+	+	+												
21	0	+	+	+												
22	Killed by bleeding for examination															

(2) *Changes in the skin caused by the ticks.*

The behaviour of biting ticks has been systematically investigated by Nuttall and his associates, and the results were published in 1908[1]. There are, however, but few records of the histological study of the actual lesions caused by ticks (Moskwin, 1929; Hoeppli and Feng, 1931), and none as far as I can ascertain which determine the fate of transmitted protozoan parasites.

The cutaneous lesions in our experiments were somewhat variable and cannot be considered as all dating exactly from the time when the ticks were placed on the ears of the calves. Some of the ticks settled down immediately while others spent about a day crawling around as if in search of a suitable place for attachment. Occasionally several abortive attempts were made at attachment before a final place was selected. The reason for this is obscure, but it is perhaps related to differences in the blood supply and in secretion of the cutaneous glands.

When a suitable place is found a cement-like substance is formed which has been briefly referred to by Hoeppli and Feng as follows: "The homogeneous eosinophilic masses which surround the rostrum extend also a short distance under the keratinised layer of the epidermis; similar eosinophilic masses were found in a few small blood vessels. The eosinophilic area around the rostrum is evidently of double nature, representing the secretion discharged by the tick and also destroyed tissue changed in its affinity to eosin under the influence of the secretion."

We are unable to agree entirely with these authors. In our preparations the cement substance can hardly be described as homogeneous and eosinophilic. That it is definitely laid down in layers and in this sense stratified is illustrated in Figs. 2, 7 and 8. Quite frequently layers which are finely granular will alternate with others which do look more or less homogeneous. Some layers are coloured red by Giemsa's stain and others bright blue. In the layers nearest to the surface of the epidermis granules of melanin can occasionally be seen, also desquamated epithelial cells, but blood cells are of rare occurrence. The fraction of the cement substance formed from degenerating epithelial cells is a small one. It is unlikely that a fluid exudate from the underlying corium is the sole factor involved, at least in the beginning, because, as indicated in Fig. 1, the corium shows no signs of injury. Later on, as the cement substance increases greatly in amount, an exudation of tissue fluid or of serum may contribute to its formation, although the cement substance which penetrates into the corium seems to be of exogenous origin, judging from the reactions of the tissue elements to it. The two remaining possibilities are: first, that the cement substance represents a local accumula-

[1] See Nuttall, Cooper and Robinson, *Parasitology*, **1**, 171–8 and Nuttall, *J. Roy. Inst. Publ. Health*, **16**, 395–7 regarding the mechanism of a tick's mouthparts and effects of tick bites. Nuttall and Strickland, *Parasitology*, **1**, 302 first demonstrated anticoagulins in ticks' salivary glands (confirmed by Pawlowsky and Stein, 1927, see References) and cite earlier work of Sabbatani (1898), who found anticoagulins in the bodies of whole ticks ground up in saline.

tion of secretion from sebaceous glands; second, that it is poured out by the tick. The participation of the sebaceous glands cannot be entirely ruled out for the reason that a few droplets can be detected in the cement substance pointing to the possible presence in it of a small amount of fat. Its lamination, however, is of a character which suggests that successive amounts are deposited locally (*i.e.* from the tick) and do not flow toward the tick from the adjacent surface of the skin. The more probable source is that also mentioned by Hoeppli and Feng; namely, some secretion or secretions produced by the tick. Here we are faced with at least three possibilities: (1) fluid regurgitated from the gut of the tick; (2) secretion of the coxal glands; and (3) secretion of the salivary glands.

Examination of serial sections of the biting ticks does not reveal the presence of any material in the digestive tract with tinctorial properties like that of the cement substance. It is highly improbable, also, that such a comparatively large volume of material could be derived from this source. The original volume of the fluid forming the cement substance was much greater than that of the cement substance in position about the chelicerae and hypostome because allowance must be made for evaporation of water from it. The peripheral part of the cement substance is dry and more or less solid, while that situated centrally, in immediate contact with the chelicerae, is of cheesy consistency as indicated by the way that the digits of the chelicerae eat into it. Some of our preparations show this much more clearly than the one represented in Fig. 1.

While the coxal glands are not very large in biting ticks, and their contents do not suggest, by similarity in staining reaction, participation in the deposition of cement substance, the evidence available does not permit us entirely to set aside this possibility. The secretion of these glands flows out of a pore situated between coxae 1 and 2 on each side of the body. According to Robinson and Davidson (1913) the secretion of the coxal glands in *Argas persicus* "only appears *after* engorgement, large droplets of clear, colourless fluid being discharged within a few seconds or minutes of the withdrawal of the proboscis of the tick from the wound." Christophers (1906) has, on the contrary, described the secretion of the coxal glands in *Ornithodorus savignyi* as occurring at the time that feeding is taking place. Just when the secretion occurs in *Rhipicephalus appendiculatus*, the form which we used, we do not know. But at the time of feeding, the salivary gland cells are very active and contain large amounts of secretion antecedents which are eosinophilic and basophilic in staining reaction.

During the 1*st day* the cement substance is only present in small amounts (Fig. 1) and the ticks may or may not succeed during this period in making an aperture in the epidermis. Until this is done there is no noticeable reaction on the part of the cells in the corium beneath.

Penetration usually occurs at some time during the 2*nd day*, and the cellular elements of the corium quickly respond. There is a swelling of the capillary

endothelium, an infiltration with polymorphonuclear leucocytes and a slight increase in eosinophiles, which later becomes very marked—a phenomenon first noted by Cleland (1910) in the exudate from a tick bite. From this point on we found it impracticable to seriate the tissue changes day by day because some ticks engorged so much more rapidly than others. Moreover, we were not always successful in orientating the pieces of skin in such a way that the microtome knife passed vertically through the aperture made by the tick and the tissue in immediate contact with the proboscis.

Fig. 2 shows that a large amount of cement substance may find its way through the wound into the corium. In this situation it spreads slowly along the lines of least resistance and does not seem to be very irritating to the cells with which it comes in contact. Fig. 5 represents part of a section made through the corium beneath the epidermis and parallel to it. It will be seen that the chelicerae and hypostome are invested in cement substance, which here also is laminated.

In an area extending at least 2 or 3 mm. on either side of the point of penetration the tissue reaction is marked (Fig. 4). The eosinophiles give place to macrophages which ingest some of the cement substance. Basophilic leucocytes, or mast cells, which normally are rather more numerous in the subcutaneous tissue than elsewhere, show a definite increase in number. The capillaries become dilated and many of them are packed with polymorphonuclear leucocytes. The polymorphonuclears are also abundant extravascularly as in the earlier stages of the reaction.

The local changes in the corium are illustrated in Fig. 3. There is a marked cytolysis of the fixed tissue cells, such as fibroblasts and histiocytes, and of the swollen endothelial walls of the capillaries, leading to haemorrhage—this is also shown in Figs. 6, 8 and 9 of sections which were not made so accurately in the path of the proboscis. The substance which causes this cytolysis is presumably of salivary gland origin, but we can find no records of its physiological study in extracts of the salivary glands which have been investigated chiefly from the point of view of their anticoagulant contents. The cytolysins destroy only the local tissue cells and do not affect the blood cells which are drawn up into the tick.

(3) *Failure to find parasites at the site of feeding.*

This examination of the skin did not reveal any structures which we feel convinced were parasites introduced by the ticks, although twenty-five of the sixty-six attached ticks were found to contain sporozoites in their salivary glands. This failure is, however, not so surprising as it would appear to be at first sight. The chances were actually not greatly in our favour. We spread our observations over the periods indicated in Table I because we wished to study the behaviour of the parasites in the ticks before, during and after possible transmission, as detailed in the first part of this paper. We desired

also to determine the sequence of tissue changes resulting from the bites of the ticks. Had we been concerned only in finding the parasites in the skin we would have ignored the first 2 days and would have concentrated our attention on removing many more pieces of skin during the 3rd, 4th and 5th days when it has been proved (Nuttall and Hindle, 1913 and others) that transmission takes place, thus multiplying our chances of finding the parasites. During and after the 6th day the likelihood of discovering parasites locally would steadily diminish (until they return to the skin by the blood stream), because one would expect them to pass on to the lymph glands which are the first organs of the body regularly involved. Some may be destroyed *in situ* by the action of phagocytes, but of this we have no evidence.

It is clear, therefore, that our search for parasites with any real prospect of success is narrowed down to the twenty-six pieces of skin removed for examination during the 3rd, 4th, 5th and 6th days after infestation with ticks. We cannot tell precisely what percentage of the corresponding twenty-six ticks contained parasites at the time when they commenced biting, but fourteen of them possessed parasites in their salivary glands at the time when they were examined. It is probable that the remaining twelve ticks were wholly devoid of parasites when they began to bite, because we observed that the ticks are not cleansed of parasites when they do bite. Instead, a considerable number of parasites remain and can be observed in the process of degeneration within the salivary glands. We can accordingly assume that twelve of the twenty-six pieces of skin did not receive any parasites. The number of skin specimens likely to be positive is thus reduced to fourteen and there are two principal reasons why parasites actually present in them could have been overlooked. The parasites in the infective sporozoite stage are so tiny that if present singly or in small masses in an extracellular position they might easily be completely decolorised in the process of differentiation of the stain. With this in mind we differentiated our sections only very slightly. In the second place, it is possible that the parasites were injected by the ticks so deeply into the subcutaneous tissue that they were not present in the comparatively superficial pieces of skin removed, which seldom had a depth from the surface of more than 3 mm. as measured in the final mounted and stained sections.

(4) *Migration of parasites to lymph glands.*

It was not possible to trace the passage of parasites from the point of entry in the skin to the lymph glands. Since the ears were the parts infested with ticks and the lymph glands at the angle of the jaw were the first to enlarge, it seems probable that the parasites travelled in the lymphatics. But when, later in the disease, other lymph glands, as for example those in the inguinal region, become involved, the parasites coming from previously infected lymph glands must reach them *via* the blood stream, because there is no direct lymphatic connection.

(5) *Kinds of cells parasitised.*

We have only found developing Koch bodies in cells of the lymphocytic series. In order that there may be no misunderstanding we wish to explain that our account of cells of this kind is based upon a tentative acceptance of the views concerning them expressed by Maximow and Bloom (1932). We shall use the terms which they employ with the meanings which they attach to them. The sort of cell chiefly parasitised is the medium-sized lymphocyte (Figs. 26, 33 and 35). Parasites are less frequently seen in small lymphocytes in which the nuclei are by volume greater than the cytoplasm (Fig. 25). But small lymphocytes become progressively less numerous in the course of the disease both in the lymph glands and in the circulating blood. It is difficult to say how many of them are destroyed and how many of them change their properties so that they become known by different names. It is possible that some enlarge and become medium-sized lymphocytes. In large lymphocytes (Figs. 34, 38 and 39) Koch bodies are also observed. That the cells illustrated in these figures are not monocytes or macrophages is shown by the large amount and the distinctive arrangement of their nuclear chromatin. Cells of this third type require a considerably longer time to find in sections, the medium-sized lymphocytes being seen immediately and the small lymphocytes after only a short delay. The Koch bodies in large lymphocytes almost invariably occupy a smaller proportion of the cytoplasm than in the other kinds of lymphocytes in the vicinity. Frequently their absolute size is also less. This seems to indicate either that the large lymphocytes are parasitised at a later stage of the disease or that the parasites develop more slowly in them. Sometimes medium-sized lymphocytes hypertrophy (Fig. 29) and look rather like large lymphocytes, but can be distinguished from them by their relatively small cytoplasmic area. Lymphoblasts (Figs. 27 and 28) constitute the fourth kind of cell of the lymphocytic series involved. Practically all of the lymphoblasts in the germ centres contain Koch bodies when the disease is first established. Like the small lymphocytes, however, they soon almost disappear, whether by degeneration or transformation we do not know. These observations are based more upon the study of sections than of smears, because topographic details are lost in the latter.

Only once have we seen parasites in a cell which looked like a polymorphonuclear leucocyte (Fig. 32). We are doubtful about the status of the cell illustrated because all undoubted leucocytes were free from them and because lymphocytic nuclei occasionally degenerate by karyorrhexis, so that the resulting masses simulate the lobes of a polymorphic nucleus (Fig. 31). The possibility remains that leucocytes ingest parasites when they are first introduced into the corium by ticks, but on this point we have no evidence. No parasites were observed within endothelial cells. In the parenchymatous cells of the liver parasites were detected after a fairly long search (Fig. 41) in two out of eleven cases which showed them elsewhere. By their staining reaction

and uniform size they could be easily distinguished from pigment and the particles of material often normally present which are coloured red by Giemsa's stain. In this situation they generally occurred within vacuoles and never in the form of developing Koch bodies. They may have been set free by the disintegration of lymphocytes containing Koch bodies which occasionally enter liver cells (Fig. 40). The absence of parasites in endothelial cells will be described later under the heading of the vascular system.

(6) *Appearance of agamonts.*

Parasites in the form of Koch bodies were first observed in lymph-gland smears of calf No. 5840 on the 13th day after infestation with ticks; calf No. 5838 on the 15th day; and calf No. 5869 on the 8th day (Table II). The fourth calf, No. 5199, died under chloroform anaesthesia on the 8th day. Although it had from the beginning of our observations a *P. mutans* infection, it might have reacted to East Coast fever at a later time. The short period of 8 days is more unusual than the longer one of 15 days. Meyer (1911) found parasites 21 days after infestation, and Gonder (1911) stated that they usually appeared about the 10th day.

The difference of a week in the response of calves Nos. 5869 and 5838 is not easily explained. The history of the ticks applied to each is given in Tables III and IV of the first paper. Both were heavily infected as nymphae and transmitted as adults. It can hardly be principally a question of the speed with which the ticks bite because both series of ticks appeared to attach themselves at about the same rate, and the time of transmission is, as Walker (1930) remarks, rather strictly limited to from 60 to 120 hours after attachment. Perhaps calf No. 5869 received a more massive dose of sporozoites than calf No. 5838, which did not exhibit parasites until a week later. Or it may be a case involving to some extent the uncertain factor termed "individual susceptibility."

The time when the rising temperature first exceeds 103° F. is a better base line than the time of infestation with ticks from which to reckon the subsequent behaviour of the parasites. Observations made in this way are listed in Table II. It will be noted that Koch bodies were first detected in lymph-gland smears during the 1st day of fever in calf No. 5838, on the 2nd day in calf No. 5869 and on the 3rd day in calf No. 5840. In addition to these observations on our standard series of calves, we noted the relation of the appearance of Koch bodies, without distinction as to their agamogenous or gamogenous nature, in gland smears to the 1st day of fever in several other animals (Table VI).

Concerning the exact stage of the parasite first to appear in the lymphocytes, Gonder (1911) writes as follows: "The first stages which are found in cattle are mostly small extra globular round forms...in the lymphatic glands or in the spleen. A few days later they are also found intracellular in the large mononuclear lymphocytes. Soon the parasite increases in size, the

number of nuclei increases considerably, and finally the parasite breaks into as many parts as there are nuclei. This is the usual way; in the case of intracellular parasites, the host cell, the lymphocyte, disintegrates earlier than the parasite."

In our lymph-gland smears taken during the 1*st day* of fever (calf No. 5869) the first parasites seen were not extracellular but were within the cytoplasm of medium-sized lymphocytes. One of them is illustrated in Fig. 9. It is a small spindle-shaped object, situated on the opposite side of the nucleus from a portion of the cytoplasm of the lymphocyte which projects as a kind of pseudopod, and it is distinctly larger than the sporozoites seen in the salivary gland cells of the biting ticks. In the photomicrograph only its spherical nucleus is visible. This nucleus is less dense and stains less intensely than do the nuclei of the sporozoites. It is the light blue staining cytoplasm in the original preparation which gives the parasite its spindle shape. The cytoplasm extends for a distance on either side of the nucleus approximately equal to the diameter of the nucleus. The parasite is contained in an area of the cytoplasm of the lymphocyte which colours distinctly less intensely than the rest. Quite frequently single parasites of this sort tend to be pear-shaped with rather more cytoplasm at one pole of the nucleus than at the other. Obviously the properties of the sporozoites are altered as soon as they enter the lymphocytes. They grow.

During the 2*nd day* of fever and sometimes in the 1st day the single nucleus increases in size, elongates and divides so that forms with two, three, four and more nuclei are met with. This is presumably the process of schizogony. It may occur in resting cells as well as in cells undergoing mitotic division. Fig. 10 represents a parasite possessed of four nuclei situated to the left of a clump of chromosomes. Gonder states that: "The propagation of the nuclei which keeps pace with the growth of the parasites is generally effected amitotically by means of successive division....I could discern (which is an exceptional case) on sections and in moist fixation an imitation of primitive mitosis, similar to coccidia and haemogregarine (karyolysus)." The latter observation we were unable to confirm. The multinucleated forms developed are typical Koch bodies. In the majority of parasitised cells at this time only one is found. When several are noted it is an indication of multiple infection. In the 2nd day of the febrile reaction Koch bodies are seen (in smears) both in the cytoplasm of the lymphocytes and extracellularly. It is a question at this early stage how many of them are actually extracellular in the lymph glands *in vivo* and how many escape from the lymphocytes as the smears are being made. Some lymphocytes are obviously destroyed in making the smears. They may be unusually fragile to begin with; but we have no satisfactory measure of this hypothetical fragility. Later on there can be no doubt that the Koch bodies are extracellular as well as intracellular.

All of the Koch bodies described thus far are properly called "agamogenous" by Gonder. They are represented as seen in sections after coloration

by Giemsa's stain in Figs. 25–29 and 33–36. Close examination of the agamonts failed to bring to light the regular presence of the second and smaller nucleus which Gonder described as occurring after schizogony and interpreted as a kind of blepharoplast. Small additional particles of chromatin are rarely seen, but they are inconstant.

Lymphocytes containing agamonts make their way out of the lymph glands into the peripheral circulation on the *3rd* and *4th days* after the temperature first exceeds 103° F., as indicated in Table II. It was on the *5th day* that parasites presumably derived from these Koch bodies were first noted in smears of red blood cells of these two calves (Nos. 5840 and 5869).

(7) *Appearance of gamonts.*

The second kind of Koch body, which Gonder styled "gamogenous," appears later. These gamonts were first seen in lymph-gland smears on the *5th day* in calf No. 5840 and the *6th day* in calf No. 5869. There was a delay between the commencement of the agamogenous and the gamogenous generations of 2 days in calf No. 5840 and of 5 days in calf No. 5869 (see Table II).

Table II. *Sequence in development of parasite; the day the temperature first exceeded* 103° *F. being called the* 1*st day.*

	Stage of parasite	Calf 5840	Calf 5869	Calf 5838
*C**	Agamonts appear in lymphocytes in gland smears	3 days	2 days	1 day
D	Reduction in number of small lymphocytes in sections of excised glands	No observations	2 „	2 days
E	Agamonts appear in lymphocytes in blood smears	4 days	3 „	Killed for tissue 3rd day
F	Parasites appear in r.b.c. in blood smears	5 „	5 „	No observations
G	Gamonts appear in lymphocytes in gland smears	5 „	6 „	„
H	Gamonts appear in lymphocytes in blood smears	7 „	7 „	„

* The letters indicate when these developments occurred in Charts I to IV.

The distinction between the two forms as seen in smears is well illustrated in Fig. 12. A little above the centre and slightly to the right a large agamogenous Koch body is visible, containing seventy-five or more nuclei, which appears to be free and unattached to any lymphocyte. In touch with it below and to the left is the irregularly outlined nucleus of a medium-sized lymphocyte, the chromatin of which is loose and not very deeply stained. The cytoplasm of this lymphocyte is almost completely filled with a large gamogenous Koch body. A comparison of the two shows: (1) that the nuclei of the agamogenous Koch body are larger, more irregular in shape and less intensely stained; (2) that the cytoplasm, on the contrary, is much more dense and deeply stained. A differentiation between the two types is readily made in sections also. Compare, for instance, the agamogenous Koch bodies represented in Figs. 26, 33 and 36 with the gamogenous ones illustrated in Figs. 30, 38 and 39.

Gonder stated further that the "agamont divides into gamonts *within* the lymphocyte"; that is to say, that free agamonts enter lymphocytes and there develop not always into more agamonts but into the later sexual phase called gamonts. "The agamont, before producing gamonts by schizogony, undergoes important nuclear changes. Frequent structures of chromidia are met with, especially in the intracellular forms. Undoubtedly this picture indicates a separation of vegetative and generative nuclear substance, as perceived in the cycle of development of many other Protozoa." But we have seen no indications of the formation of chromidia. Neither have we been able to distinguish two kinds of nuclear material. Thinking that the Giemsa's stain, which we regularly employed, might not bring to light this difference even though it existed, we applied the Feulgen reaction for thymonucleic acid as detailed by Cowdry (1928), because Lucas (1930) found that by this technique the two kinds of nuclear material can be readily differentiated in several parasitic Protozoa. Even by this method we were unsuccessful in confirming Gonder's observation. All we can distinguish in Giemsa and Feulgen preparations is an apparently unbroken series of gradations between the large agamonts and the smaller gamonts.

We made, however, an observation not reported by Gonder or others after him, namely, that in a single Koch body both agamonts and gamonts occasionally exist side by side. This condition is illustrated by the uppermost lymphocyte in Fig. 11. Examination shows that some of the nuclei are large and irregular and indistinguishable from those in the agamogenous Koch body represented in Fig. 12, while others are small and apparently similar to gamogenous forms likewise shown in Fig. 12. Such Koch bodies we call "mixed." Their incidence is definitely less than that of the pure agamogenous and gamogenous varieties.

(8) *Lesions in lymph glands.*

It was only by the study of sections that we could hope to determine the structural changes in the lymph glands which accompany the parasites. As a basis from which to estimate the extent of the alterations we employed preparations of lymph glands of normal calf No. 5837 which had been reared free from tick-borne diseases. To ascertain the earliest lesions lymph glands removed under chloroform anaesthesia from calves No. 5838 and 5869 were examined. The glands were excised from the former daily from the 6th to the 21st day, and from the latter daily from the 8th to the 18th day after infestation. The temperature of calf No. 5838 first exceeded 103° F. on the 14th day, but it reached 103° F. on the 3rd, 8th, 9th, 11th and 12th days (Chart III), while that of calf No. 5869 exceeded 103° F. on the 5th day and remained high (Chart II).

The first changes were noted in sections of excised lymph glands of calf No. 5869 on the 7th day after infestation (2 days of fever), and of calf No. 5838 on the 16th day after infestation (likewise 2 days of fever), when both con-

tained a few Koch bodies, but the alterations were slight and barely noticeable. They consisted only of a slight reduction in the number of small lymphocytes which it was almost impossible to measure quantitatively in the sections. We did not consider it necessary to correlate this reduction with possible changes in the number of lymphocytes in the circulating blood, because Steck (1928) has supplied detailed information in respect to the latter. He has shown that the circulating lymphocytes often begin to decrease in number before the temperature of the animals rises above 103° F., and that they continue to decrease progressively until death. More noticeable lesions are

Table III. *Distribution of Koch bodies as seen in sections.*

Tissue	Calf 5838	Calf 5840	Calf 5869	Calf 7539	Bovine 6057	Bovine 6073	Bovine 6927	Bovine 7600	Calf 7613	Bovine 7644	Bovine 7837	Bovine 7843
Adrenals	−	.	.	+++	+	+	−	+	−	+	+	−
Bone marrow	−	++	++	+++	+	+	.	−	.	−	++	− (3)
Caecum	−	+++	++	+++	++	+	−	++	−	−	++	− (4)
Cardiac muscle	−	−	.	−	+++	++	−	+++	−	++	+	++
Cerebral cortex	−	−	−	+	+	−	−	−	−	−	−	−
Colon	.	++	+	.	+	+	.	+	.	+	++	++
Conjunctiva	.	.	.	.	.	.	.	+	.	.	.	.
Cornea	.	.	.	.	.	+	.	.	.	−	.	.
Epididymis	−	.	.	.	.	.	.	.	.	.	.	.
Gall bladder	.	++	+	+++	+++	+	−	++	−	++	.	−
Kidney	−	+	++	+++	+++	++	−	++	+++	++	++	++
Liver	+	++	++	+++	+++	++	−	+++	+	++	+++	+
Lung	−	+	++	+++	++	++	−	+	++	+	+	+
Lymph glands	+	+++	+++	+++	++	++	.	++	+	++	+	+
Ovary	.	.	++	.	.	.	.	.	.	.	.	.
Pancreas	−	.	.	+++	.	+	−	.	−	.	.	−
Salivary glands	−	−	+	.	++	++	−	+	−	++	+	−
Skeletal muscle	−	−	−	++	.	+	−	+++	−	++	−	−
Skin	.	−	+	+	++	++	−	+	−	++ (1)	−	−
Small intestine	.	−	++	+++	++	++	−	++	.	+	+	+
Spleen	+	+++	+++	+++	+++	.	−	+++	+++	++	+ (2)	+
Stomach (abomasum)	−	−	−	+++	.	+	−	.	−	−	−	+
Testes	−	++	.	.	.	.	.	.	.	.	.	.
Thymus	−	+++	+	.	.	.	.	.	.	.	.	.
Thyroid	.	−	+	+++	.	−	−	++	.	−	.	+
Tongue	.	.	.	+	+	+	−	+	−	+	+	−
Ureter	.	.	.	.	.	+++	.	.	.	.	.	.
Urinary bladder	.	.	−	.	.	.	.	+	.	.	.	.
Uterus	.	.	+	.	.	.	.	.	.	.	.	.

− = no Koch bodies seen.
+ = Koch bodies seen after long search.
++ = maximum number five per field (objective 2 mm., ocular 10).
+++ = more than five per field.

(1) *Rickettsia*-like bodies observed in endothelium.
(2) bacteria numerous.
(3) tissue mostly fatty.
(4) many coccidium-like Protozoa.

illustrated in Figs. 13–16, which represents the appearance of lymph glands in calves killed *in extremis* when the number of Koch bodies was maximum. Fig. 13 is of the cortical portion of an anterior mediastinal lymph gland taken from calf No. 5838 killed on the 16th day after infestation with ticks and the 3rd day after its temperature rose above 103° F. (see Chart III). It shows a portion of a lymphoid follicle with germinal centre a little below and to the left. Above and to the right can be seen a part of the peripheral lymph sinus. The chief visible deviations from normal are the slight increase in extent of the centre and the decrease in the number of lymphocytes between

it and the peripheral sinus. Alterations in the medulla are indicated in Figs. 14, 15 and 16. They are similar to those seen in the cortex, but perhaps a little more marked in degree.

In other calves of our series the lesions were of longer standing and approximated more closely to those studied in bovines as represented in Figs. 17–20. Fig. 17 shows part of the cortex of a lymph gland taken from bovine No. 7600, 22 days after exposure to infected ticks and 11 days after the first rise in temperature. The animal was sacrificed *in extremis*. This figure, illustrating the maximum cortical change observed, should be compared with Fig. 13. Both show the relation between a germinal centre and a peripheral lymph sinus. It will be noted: (1) That the nuclei of the cells inhabiting the germinal centre are shrunken, picnotic and irregular, the lymphoblasts being replaced by lymphocytes and leucocytes. (2) That the tissue between the germinal centre on the right and the sinus on the left is greatly reduced in extent and contains few if any lymphocytes. (3) That the peripheral sinus is greatly dilated, that its walls are more cellular and that many of the cells stretch across the lumen. In describing the lesions in lymph glands, Steck states that: "In some cases there is a moderate desquamation of cells of endothelial habitus into the margina and medullary sinuses." It is important to note that the cells in question are not strictly endothelial in nature but differ from them in many ways as described by Maximow and Bloom.

Fig. 18 is from a section of a lymph gland of bovine No. 7644, also 22 *days* after infestation with ticks and 11 *days* after the first rise in temperature, which was likewise slaughtered *in extremis*. A portion of the medulla is shown. It can be seen that the endothelium of the small blood vessels and capillaries is very prominent. In some of our specimens it is more so than is indicated in the figure.

Fig. 19 shows another portion of a lymph gland from the same bovine. In about the middle is illustrated a germinal centre in which, like that represented in Fig. 17, the usual large pale-staining cells (lymphoblasts), active in the production of lymphocytes, are replaced by deeply staining ones, many of which are picnotic and degenerating.

The condition presented in Fig. 20 is significant. It shows the development of connective tissue and the almost complete disappearance of lymphocytes. Interstitial fibrosis is often more marked than is here illustrated. In summary, it is clear that the lymph glands, though swollen in advanced cases of East Coast fever, are oedematous and very poor in lymphocytes. In these terminal stages there is sometimes but not always a sharp decrease in number of lymphocytes carrying Koch bodies, specially those of the agamogenous variety.

In order to measure the incidence of Koch bodies and to ascertain whether any relation exists between their presence and cell division, definite counts were made. A ruled square was inserted in one of a pair of No. 10 compensating oculars of a Zeiss binocular microscope. The objective employed was a

2 mm. apochromatic of 1·30 aperture. The dimensions of the square were determined by the observation of a micrometer slide with the same optical system. They were $82 \times 82 \mu$. The volume of the tissue under observation in each square was $82 \times 82 \times 5$ (the thickness in μ of these particular sections) which amounts to 33,620 c.μ.

The following were counted in a sufficient number of areas covered by the square to yield approximately 1000 lymphocytes: (1) resting lymphocytes + Koch bodies; (2) resting lymphocytes − Koch bodies; (3) dividing lymphocytes + Koch bodies; (4) dividing lymphocytes − Koch bodies; and (5) extracellular Koch bodies. The results are presented in Table IV. Under the heading

Table IV. *Incidence of Koch bodies in lymph glands.*

	Calf No. 5840			Calf No. 5869			Bovine No. 6657			Bovine No. 7837		
	No.	%	Per c.mm.	No.	%	Per c.mm.	No.	%	Per c.mm.	No.	%	Per c.mm.
Resting lymphocytes +Koch bodies	267	26·40	294,137	561	55·16	834,324	129	11·66	153,486	77	7·56	72,408
Resting lymphocytes −Koch bodies	735	72·70	809,703	434	42·67	645,464	887	86·96	1055,324	930	91·35	864,533
Dividing lymphocytes +Koch bodies	4	0·39	4,406	13	1·27	19,333	2	0·19	2,379	4	0·39	3,717
Dividing lymphocytes −Koch bodies	5	0·49	5,508	9	0·88	13,384	2	0·19	2,379	7	0·68	6,506
Extracellular Koch bodies	45	—	—	3	—	—	1	—	—	0	—	—

of each animal there are three columns of figures. In the first is the number of the particular kind of cells counted except that the lowest line of figures refers to extracellular Koch bodies, not to cells; in the second is the number given as a percentage of the total number of cells counted as listed in the first four lines; while in the third the calculated number per cubic mm. of tissue is inserted. Records were kept of the number of squares of tissue examined in making determinations for each animal, namely 27, 20, 25 and 32, in the order listed in the table.

As an example, the number of cells per c.mm. represented by the 267 cells containing Koch bodies given in the upper left corner of Table IV was derived as follows: The total volume of tissue examined was the volume of a single square (33,620 c.μ) multiplied by the number of squares (27) amounting to 907,740 c.μ. If 907,740 c.μ contain 267 cells, then 1,000,000,000 (1 c.mm.) must contain X number of cells in the following formula:

$$267 : 907{,}740 :: X : 1{,}000{,}000{,}000.$$

Solving for X,

$$907{,}740X = 267{,}000{,}000{,}000 \text{ and } X = 294{,}137.$$

1 c.mm., therefore, contains 294,137 lymphocytes. The proviso must be made, however, that the c.mm. is the sum of a series of planes 5μ thick. It does not follow that if it were possible to count the cells in a solid mass of tissue, 1 c.mm. in size, that the same number of lymphocytes would be found, for it would theoretically not be necessary ever to count the same cell twice, as

would certainly frequently happen when the same block of tissue is cut into serial sections. Evidently the actual number per c.mm. would be somewhat less than our calculated number. The following precautions were taken:

(1) The sections were all made from well-fixed material and had been satisfactorily stained so that all the Koch bodies present were revealed. The observations were restricted to sections cut fairly deeply through the blocks of tissue where the cells had been less disturbed than at the surface in the manipulation of the tissue before fixation. We are satisfied that the counts are complete for calves Nos. 5840 and 5869, which were killed at the height of the reaction of the lymph glands. The lesions in them were like those depicted in Figs. 13–16. But in the case of bovines Nos. 6657 and 7837 the lesions were much farther advanced and resembled those shown in Figs. 17–20. Many of the lymphocytes had disappeared, and a large proportion of those which remained were degenerating along with the contained Koch bodies. All of the Koch bodies which could be identified by their position in the lymphocytes, size and staining reaction were enumerated. A few of them were so degenerate that their nuclei failed to stain red in the usual way when treated with Giemsa's mixture. It is likely that some were completely destroyed.

(2) In counting the cells it was not feasible to classify separately the small, medium and large lymphocytes and the lymphoblasts. They are all listed under the collective term of "lymphocytes." Polymorphonuclear leucocytes of the three varieties, endothelial cells, typical histiocytes and fibroblasts were readily excluded. Mononuclear leucocytes, or monocytes, are not usually recognisable in lymph glands. Their possible presence and consequent counting with the lymphocytes would not greatly complicate matters. Care was taken to examine in each gland approximately equal amounts of cortex and medulla.

(3) It was not always an easy matter to decide which lymphocytes were dividing and which were degenerating. As long as the lymphocytic nature of the latter was clear they were grouped with the "resting lymphocytes." Only those in which some hint of chromosome formation could be seen were classified as "dividing lymphocytes." The observance of this convention led to the omission from the group called "dividing" of some prophases and perhaps of a few telophases, but it was thought that the observations would thereby be rendered more precise. The actual proportion of dividing cells is consequently slightly higher than that given in the table.

(4) Difficulty was experienced in determining with certainty which Koch bodies were extracellular. Only those which stood apart from lymphocytic nuclei were so listed. The possibility remains, however, that some of these were contained in parts of the cytoplasm of lymphocytes projecting into the plane of the section while their nuclei were in the preceding or following section.

Taking into consideration the glands of the four animals (Table IV), 4020 resting lymphocytes were examined, of which 1034 or 25·69 per cent. contained Koch bodies. The highest individual incidence of Koch bodies proved

to be 55·16 per cent. and was found in calf No. 5869[1], while the least was 7·56 per cent. in bovine No. 7837. As already remarked, the lesions in the two calves were less advanced than in the bovines. In the bovines they were characterised by reduction in number of lymphocytes and degeneration of the Koch bodies, but the number of animals studied quantitatively is too few to permit a conclusion.

The decrease in lymphocytes with and without Koch bodies, resting and dividing, per c.mm. of lymph gland is also indicated in Table IV. It cannot be wholly attributed to failure in the production of lymphocytes because, though there are fewer dividing cells in the bovines than in the calves, the difference is not marked. That it is partly due to local degeneration and disintegration of lymphocytes in the lymph glands is suggested by the large number of degenerating lymphocytes seen. It is questionable how many of them are swept away in the efferent lymphatic channels from the glands and eventually gain the blood stream to be cytolysed therein or to be removed by the blood filters, the spleen and the liver. If a large proportion of the disappearing lymphocytes left the lymph glands in this way one would look for a temporary increase in lymphocytes in the blood stream. Judging from the counts of Steck this never takes place. But he did find a pronounced stagnation of lymphocytes in the vessels of the internal organs, particularly the liver where they accumulate in numbers sometimes far exceeding the normal. This redistribution of lymphocytes is, however, not sufficiently massive to explain, of itself, their disappearance from the lymph glands.

It would appear that a hyperplasia of lymphocytes in the lymph glands, if it occurs at all as a result of the presence of parasites, is very temporary. In no case did the percentage of dividing lymphocytes exceed that observed in our normal control calf. In these four cases of East Coast fever which we investigated quantitatively the proportion of dividing lymphocytes which contained Koch bodies was about the same as that of lymphocytes devoid of parasites. The available evidence seems to indicate that the swelling of lymph glands, which is so characteristic a symptom of East Coast fever, is not caused by hyperplasia but rather by the accumulation of fluid, in other words, by oedema.

We did not find as many extracellular Koch bodies as we anticipated. Their number in the specimens studied quantitatively was so small that one would not be inclined to attach much significance to this reputed phase of the parasite in lymph glands were it not that in another animal (bovine No. 7538) they were very numerous, and for their abundance in some lymph gland and blood smears. There is reason to believe that some at least of the Koch bodies only escape from the lymphocytes when the latter disintegrate. The Koch bodies often develop within lymphocytes in close association with the centrosomal apparatus which is situated on that side of the nucleus provided with most cytoplasm. When the nucleus is indented it occupies the

[1] In another bovine (No. 7538), however, we observed an incidence of roughly 90 per cent.

concavity. The centrosomal apparatus is more resistant to the action of the parasite than the rest of the cytoplasm. It often retains its basophilic properties even when the cytoplasm is almost completely occupied by a Koch body or bodies (Figs. 30–32). Gonder shows it in some of his illustrations but does not describe it. In our opinion the presence of traces of this centrosomal apparatus in apparently free Koch bodies is proof that they originated within cells. The converse, that the absence of microscopically visible remains of this cytoplasmic component indicates an extracellular origin, does not hold because it may have completely degenerated or may not have been incorporated within the Koch body, for some of them develop apart from the area presumably occupied by the centrosomal apparatus, especially in large lymphocytes (see Fig. 39). Unfortunately we did not fix our specimens in fluids best adapted for the demonstration of centrosomes.

(9) *Blood-forming organs.*

Under this heading are included the bone marrow, the spleen and the liver. In the *bone marrow* of the femur and humerus, K. F. Meyer (1911) found in a single animal which died 28 days after infestation with ticks that the cells containing Koch bodies numbered one for every two microscopic fields, while in the ribs there were three and four cells per field. We observed them in the bone marrow of six of twelve animals as indicated in Table III. Our microscopic fields included less tissue than those of Meyer because we employed a No. 10 ocular while he used a No. 4. Equal counts, therefore, mean that the parasites were much more numerous in our specimens than in his. We were not successful in observing the haemorrhages in the yellow marrow, which he reported (though from the frequency of haemorrhages in other parts of the body they probably occur in some cases), or any change in the bone marrow which could be considered as pathognomonic. But the fact that Steck observed in the peripheral blood a decrease of leucocytes as well as of lymphocytes would indicate that the marrow is affected.

In the *spleen* we found Koch bodies in all of ten cases and again very much more abundantly than did Meyer. In only one autopsy was a swelling of the spleen noted.

The *liver* exhibited marked perivascular lymphocytic infiltrations, but it remains to be discovered how far the infiltrations antedate the East Coast fever reaction and to what degree they are definitely attributable to it. It is logical to assume that if pre-existing they may be greatly accentuated as a result of the disease.

The lymphocytes which make up the infiltrations often possess Koch bodies (Figs. 21, 38 and 39). They were studied intensively by the methods described under the heading of lymph glands. This was done in the hope of being able to ascertain whether the cells invaded the tissue from the blood stream, the path being prepared by injury to the endothelium and small haemorrhages as claimed by K. F. Meyer. Obviously the only infiltrations in which the cells

could be quantitatively studied were those of sufficient extent to allow the superposition of the ruled square over the infiltrated area existing between the wall of the vessel and the parenchyma of the organ. The results are expressed in Table V.

Table V. *Incidence of Koch bodies in perivascular infiltrations in the liver.*

	Calf No. 5840			Calf No. 5869			Bovine No. 6657			Bovine No. 7837		
	No.	%	Per c.mm.	No.	%	Per c.mm.	No.	%	Per c.mm.	No.	%	Per c.mm.
Resting lymphocytes + Koch bodies	439	42·17	567,726	359	34·10	533,908	467	44·94	534,251	625	62·31	929,506
Resting lymphocytes − Koch bodies	585	56·19	756,538	681	64·66	1012,775	554	53·32	633,780	367	36·59	545,806
Dividing lymphocytes + Koch bodies	12	1·15	15,518	11	1·04	16,359	11	1·05	12,584	9	0·89	13,221
Dividing lymphocytes − Koch bodies	5	0·48	6,466	2	0·19	2,974	7	0·67	8,008	2	0·19	2,974
Extracellular Koch bodies	11	—	—	3	—	—	23	—	—	15	—	—

To economise space the contents of Table V will be allowed to speak for themselves; they need not be repeated in the text. It will suffice to emphasise two points: (1) That in this case it is the two bovines which exhibit the highest percentage of cells containing Koch bodies whereas in the lymph glands it was the calves which showed the highest percentage. This observation suggests that in the bovines the brunt of the injury is borne by the lymph glands and that the influence causing the destruction of lymphocytes, whatever it may be, does not act so forcibly in the liver in which many cells contain Koch bodies which show by contrast little if any sign of degeneration. (2) That in the livers of both calves and bovines the dividing cells containing Koch bodies exceed those devoid of them in the proportion of 43 to 16, though the resting cells devoid of Koch bodies are less numerous than those provided with them in the proportion of 1890 to 2187. This may mean that the presence of Koch bodies in the lymphocytes is, in the liver, a stimulus to cell division, but the numbers are too small to justify a conclusion. We are therefore unable either to confirm or refute Steck's assumption that the infection of lymphocytes with the parasite is the cause of the proliferation.

The infiltrations always occurred in the perilobular spaces in association with the hepatic artery, portal vein, and bile ducts. The neighbouring liver cells were not regularly injured to any noticeable degree, though they occasionally contained lymphocytes provided with Koch bodies (Fig. 40) and the Koch bodies rarely escaped and disintegrated in the cytoplasm (Fig. 41); but the endothelium of the sinusoids was in some cases swollen and the Kupffer cells prominent.

(10) *Vascular system.*

Examination of our autopsy reports yields the following information concerning the heart and blood vessels:

Bovine No. 5922—(a recovered animal).

Bovine No. 6657. Epicardial petechiae; haemorrhages in renal cortex.

Bovine No. 6673. Some lymph glands hyperaemic or haemorrhagic. Haemorrhages in gastric omentum, mesentery, serosa of small intestine, pleura costalis and epicardium. Slight ecchymosis left endocardium. Subscapular haemorrhages in spleen. Haemorrhages rare in pharyngeal mucous membrane and sides and ventral surface of tongue. Haemorrhages in mucosa of large and small intestine. Blood coagula in lumina of colon, caecum and rectum. Haemorrhages in mucous membrane and serosa of gall and urinary bladders. Haemorrhages on surface and within calyx of kidneys.

Bovine No. 6927. Ecchymoses in left endocardium (a recovered animal).

Calf. No. 7539. Endocardial ecchymoses.

Bovine No. 7600. Slight icterus renal cortex. Patchy hyperaemia of duodenum, ileum and caecum and hyperaemia of colon.

Calf No. 7613. Haemorrhages in fourth stomach, caecum and rectum. Ileocaecal valve haemorrhagic.

Bovine No. 7644. Hyperaemia of lung and small intestine. Epicardial and endocardial haemorrhages. Haemorrhages in stomach, colon, gall bladder and some lymph glands.

Bovine No. 7837. Slight hyperaemia of lung. Haemorrhages of calyx major of kidney and bone marrow of femur.

Bovine No. 7843. Haemorrhagic foci in lungs. Rare pin-point haemorrhages in kidneys. Lymph glands rarely haemorrhagic.

Evidently the vascular system is more definitely involved than would appear from the study of sections alone. An analysis of autopsy reports on sixteen animals by Sir Arnold Theiler (1911) shows that epicardial or endocardial haemorrhages or both were noted in thirteen; that eleven exhibited slight haemorrhages in other parts of the body, and that in only one animal were no haemorrhages seen. Sergent *et al.* (1927) found epicardial petechiae, which were sometimes accompanied by endothelial ecchymoses, in six animals infected in Algiers by ticks, sent to them from South Africa by Sir Arnold Theiler and Dr du Toit. The reports published by Steck relate to fourteen animals. He found epicardial or endocardial haemorrhages or both in six, haemorrhages in other parts of the body in three and no visible haemorrhages in five. K. F. Meyer was probably justified in emphasising the occurrence of small haemorrhages. But even so the haemorrhages in our specimens are not sufficiently widespread to offer paths for the migration of lymphocytes from the blood stream into the perivascular spaces in accordance with his hypothesis. In our small series we were not able to establish any quantitative relation between the frequency of haemorrhage seen at autopsy and the degree of perivascular infiltration observed in sections. The infiltrations were more developed in bovine No. 6657, which showed comparatively few haemorrhages, than in bovine No. 6673, in which the haemorrhages were most pronounced. Though the epicardium and endocardium appear to be particularly liable to haemorrhages, the infiltrations are most marked in the kidneys and liver where haemorrhages are less pronounced.

Like Steck we failed to find the uniform swelling of the intima cells of the capillaries which Meyer reported as always present in "the kidney, liver, lungs, etc." In another place he refers to the intima cells of "several capillaries" of the bone marrow as projecting into the lumen. The impression is given by his discussion and by the use of the abbreviation "etc." that he believed this swelling to be of widespread occurrence and of considerable significance (see also his 1930 account). By "intima cells" he clearly means endothelial cells. The cells lining the sinusoids of the liver are not typical endothelial cells but highly specialised ones. We agree with Meyer in so far that in some cases we have found the endothelial cells of these sinusoids swollen and the Kupffer cells prominent, as we have already noted. In lymph glands we have observed and illustrated (Fig. 18) slight swelling of typical vascular endothelial cells. The cells lining the peripheral lymphatic sinuses which (with Steck) we have observed in the process of desquamation are not, properly speaking, endothelial cells, but rather "littoral cells" in the sense of Maximow and Bloom. Since we did not study scarpings or sections of the larger vessels we are not in a position to check Meyer's statement that the endothelial cells in this situation contain Koch bodies. We can say, however, that though we examined many small muscular arteries, arterioles, capillaries, venules and small veins in our sections we never observed a single definite case of Koch bodies within endothelial cells. The absence of Koch bodies in the lining endothelium is the more significant because the blood is often swarming with parasites.

In the heart muscle lymphocytes containing Koch bodies were noted in six out of eleven animals examined in the acute disease (see Fig. 24 and Table III). They were absent in two recovered animals (bovines No. 5922 and 6927). The muscle fibres were slightly degenerated in the neighbourhood of areas of infiltration in bovine No. 6673.

(11) *Endocrines.*

The only endocrines consistently studied were the adrenals, the islets of Langerhans of the pancreas, the thyroid and the thymus. The *adrenals* did not exhibit noticeable lesions at autopsy, but sections from six out of nine adrenals showed extravascular lymphocytes with Koch bodies. Usually they were sparsely distributed, but in one case (calf No. 7539) they were abundant (more than five per field). K. F. Meyer found 1–20 per field. The lymphocytic infiltrations observed while never extensive in the adrenals were in them, and in most of the organs investigated, of two types. The first and most common kind consisted of accumulations of lymphocytes in and about the adventitia of small vessels which were fairly definitely circumscribed and did not extend far into the surrounding tissue. The second, seen rather less frequently, was focal in type, rather more extensive, seemed to invade the adjacent parenchyma causing slight destruction of cells and probably originated in association with a blood vessel of some kind.

The *islets of Langerhans* were studied in eight animals, two of which had recovered from the disease. They did not exhibit any distinctive alterations.

The *thyroid* was examined in ten animals, two of which had recovered. Koch bodies were noted in calf No. 5869, bovine No. 7600, bovine No. 7843 and calf No. 7539. In the last named they were very numerous (see Table III). Perivascular infiltrations were present but were never highly developed.

Particular interest attaches to the *thymus* because the so-called small round cells (thymocytes) in it are thought by many to be lymphocytes. Unfortunately our study of the thymus is restricted to calves No. 5840, 5869 and 5838. No thymic vestiges were secured from the bovines. The organ as a whole is but little modified. The oedema, destruction of lymphocytes and fibrosis so characteristic of lymph glands are absent. The small round cells are not invaded by the parasite which occupied cells which look like medium-sized lymphocytes. These are most abundant in the cortex though many are found in the medulla.

(12) *Alimentary tract and pancreas.*

It is well known that the alimentary tract is seriously injured in East Coast fever. The haemorrhages have been mentioned. Bovine No. 6657 showed gastro-enteritis, calf No. 7539 ulcerative gastro-enteritis, bovine No. 7600 oedema of the fourth stomach, bovine No. 7644 ulcerative gastritis and oedema of stomach, bovine No. 7837 oedema of caecum and colon and bovine No. 7843 infiltration of folds of fourth stomach and walls of caecum and colon. But bovine No. 6673 and calf No. 7613 exhibited none of these changes though they suffered severe attacks of East Coast fever and were killed *in extremis*. The relation of changes of this sort to typical East Coast fever is uncertain. The ulcerative lesions may have preceded the East Coast fever, while the oedema, which may have been a more acute process, may have been caused by it.

Since there are normally many lymphocytes in the mucosa of the alimentary tract, particularly in its lower part, it is natural to expect microscopic changes in it. The number of these lymphocytes is greatly increased in East Coast fever and a very large proportion of the medium-sized ones contain Koch bodies. Data on this point are given in Table III. The lymphocytes often accumulate perivascularly and in foci as mentioned under the heading of the adrenals. The epithelial cells are, however, not noticeably injured thereby.

The *pancreas* is probably as little altered as any other epithelial organ. Koch bodies were detected in it in only three animals. They did not accompany well-developed perivascular infiltrations.

(13) *Urino-genital system.*

Lesions in the kidneys have been frequently described by other investigators. Gray and Robertson (1903) and Koch (1903) reported the discovery of elevations on the surface of the kidney which they regarded as infarcts.

Owing to their light colour they have also been spoken of as "white foci." They vary in size from the lower limit of naked eye visibility to about 1 cm. in diameter. Collaud (1906) and Martin Mayer (1910) reached the conclusion, however, that they are not infarcts but are of inflammatory nature. K. F. Meyer (1911) also failed to find evidence supporting the infarct theory and developed the hypothesis already mentioned that as a result of small haemorrhages Koch bodies are washed into perivascular situations where the lymphocytes containing them multiply. He says: "Such a focus grows larger and resembles more or less a lymphoma or a metastasis of a growth." Steck (1928) does not agree with K. F. Meyer, for he failed to find the vascular changes reported by Meyer. According to Steck: "...the whole focus is nothing but a rapidly proliferating lymph follicle and it is not surprising that it should develop where such structures normally take their origin...in the adventitia of the small arteries."

Before describing our findings we shall mention briefly the evidence bearing on the incidence of these "white foci." It is recognised that they are not invariably met with in cases of East Coast fever. On the basis of wide experience James Walker (1930) states that the most characteristic lesions are in the kidneys. They consist of "red or pale yellowish white spots, varying in size from a pin-head to a pea...known as infarcts. They are not present in all, but in a high percentage of cases." Sir Arnold Theiler (1911) found "infarcts" in seven out of sixteen cases; Sergent *et al.* (1927) noted them constantly in six cases; and Steck (1928) noted "lymphoid foci" in one out of fourteen cases; while we observed them, likewise at autopsy, in five out of ten cases.

We accept the interpretation of the "white foci" given by Steck with the qualification that the accumulation of lymphocytes and mononuclear leucocytes can hardly be regarded as a typical lymph follicle from which it differs by the presence of Koch bodies, the way it invades the surrounding tissue and the frequency of evidences of cell degeneration which can be seen in it. The fact that typical cases of East Coast fever occur in which none of the white foci are recognisable at autopsy may be significant. When, however, the kidneys are examined microscopically the vast majority (ten out of twelve of our typical cases) show focal and perivascular infiltrations of variable extent (Fig. 23). It may be simply a question either of the more rapid development of the foci in a minority of the animals or of the pre-existence of lesions on the basis of which large foci, easily visible to the naked eye, are more rapidly formed.

Our specimens of other parts of the urinary system are few. We have examined only the urinary bladder of calf No. 5869 and of bovine No. 7600 (Fig. 22), and the ureter of bovine No. 6673. Koch bodies were only noted in the wall of the urinary bladder of bovine No. 7600 and in the wall of the ureter of bovine No. 6673. They were accompanied by slight perivascular infiltration.

The *testicles* of calf No. 5840 exhibited slight infiltrations and a moderate

number of Koch bodies, while in those of calf No. 5838, in which the lesions in other tissues were much less noticeable, neither were observed. The *ovaries* of calf No. 5869 showed infiltrative conditions comparable to those in the testicles of calf No. 5840 (see Table III). In the only uterus examined (calf No. 5869) the infiltrations were likewise slight and the Koch bodies moderate in number. K. F. Meyer has observed Koch bodies in the foetus, both intra-uterine and *post partum*.

(14) *Respiratory system.*

Hyperaemia and oedema of the lungs seem to be as characteristic autopsy findings in East Coast fever as "white foci" in the kidneys. Theiler found hyperaemia in five and oedema in twelve out of sixteen cases, Sergent *et al.* (1927) oedema in four out of six cases, while Steck noted hyperaemia in one and oedema in seven out of fourteen cases. In our series of ten autopsies hyperaemia was observed in four and oedema in three. One of them exhibited hyperaemia without oedema; but Theiler and Steck found hyperaemia only in the oedematous animals. Microscopic study of our sections of lungs revealed considerable lymphocytic infiltration, but this was not as marked or of as regular occurrence as in the liver and kidneys. K. F. Meyer found by detailed examination of a single case that Koch bodies "were fairly rare, about 1–20 (microscopic) fields." We discovered Koch bodies in ten out of twelve animals, but two of the latter had recovered from the disease and failed to show Koch bodies in any other tissues. The maximum number noted was five per field.

(15) *Nervous system.*

Both lesions and Koch bodies are seldom found in the nervous system. We observed Koch bodies in the cerebral cortex in only two out of twelve animals which showed them in other parts of the body. Accompanying these Koch bodies were very slight infiltrations.

(16) *Blood stream.*

The time of appearance of agamonts and gamonts in lymphocytes in the blood stream has already been referred to (see also Tables I and II). Further data are given in Table VI which need not be recapitulated in the text except to state that, on the average: (1) The temperature first surpassed 103° F. on the 9·8th day after infestation with ticks. (2) The first Koch bodies within lymphocytes were observed on the 14·8th day, that is to say, 5 days after the fever commenced[1]. (3) The first parasites within the red cells were detected 1·6 days later on the 16·4th day after infestation.

Parasites were studied in the red cells of twenty-two animals, of which calves Nos. 5723 and 5740, mentioned in the first paper, and calves Nos. 5840, 7539 and 7613 described herein, constituted the most valuable material

[1] On the basis of individual cases and not of averages they were first seen the day the fever first exceeded 103° F. (Table II).

because they were pure experimental infections uncomplicated by other tick borne diseases. Among the adult bovines, Nos. 4810, 5759, 5760, 5774, 6673, 7600 and 7644 proved particularly interesting though all of them were naturally infected with *T. parva* and most of them had probably recovered from various tick borne diseases.

We first examined the parasites in red cells intensively in calf No. 5840 by the daily examination of smears of blood taken from the ear. The resulting

Table VI. *Time in days, after tick infestation of bovine, when observed first rise in temperature and appearance of Koch bodies and parasites in red blood cells.*

Animal	Temp. first exceeded 103° F.	Days	Max. temp. ° F.	First Koch bodies in gland smears	First parasites in red blood cells
Bovine 3519*	11	20	106°	16	16
Calf 5259	10	11	106·6°	13	18
Calf 5430	1	18	106·6°	14	15
Calf 5446	1	11	107·4°	11	18
Calf 5586	9	13	106·6°	14	15
Calf 5723	12	18	107°	16	17
Calf 5740	8	14	107·4°	13	17
Calf 5840	10	15	107·4°	13	15
Calf 5869	6	10	107°	8	9
Bovine 5922*	11	20	105·4°	21	23
					Recovered
Bovine 6657*	14	20	106·6°	16	17
Bovine 6673*	12	15	106·4°	15	15
Calf 7539	9	13	106·4°	14	15
Bovine 7600*	12	14	106·2°	14	14
Calf 7613	19	24	106·4°	27	26
Bovine 7644*	12	17	105·6°	15	15
Average	9·8	15·8	106·5°	14·8	16·4

* Naturally infected animals. The time is reckoned from the day that they were placed in a locality heavily infested with infected ticks. The other animals were infected experimentally by placing ticks on their ears.

Table VII. *Parasites in red blood cells of calf No.* 5840.

Days after infestation with ticks	16	17	18	19	20	21	22
Number of reds examined	3134	1042	2071	2190	2250	2143	2071
Total number containing parasites	33	31	120	126	151	139	202
Number containing two parasites	3	6	6	15	13	11	15
Number containing three parasites	—	—	—	—	—	—	2
Number containing a constricted chromatin mass	—	1	3	5	6	5	2
Number containing two chromatin masses	—	1	3	5	9	4	3
Number showing "cross" forms	—	—	—	—	1	1	—

counts are listed in Table VII and the forms observed are illustrated in Figs. 42–76. It is important to note that in this calf the number of reds carrying parasites never reached 10 per cent. and that it was killed for the collection of tissues when the disease was clinically well established, but before the animal was *in extremis*, so that the later stages of the parasite may not have had sufficient time to develop; but the condition was a pure experimental East Coast fever infection without contamination by other piroplasms or anaplasms.

Parasites were first noted on the 15*th day* after infestation with ticks but

they were of very rare occurrence. They exhibited apparently exactly the same appearance as on the 16*th day* (Fig. 42), being indistinguishable from the single gamonts. In form they were spherical or slightly ovoid and it was usually impossible to make out any cytoplasm. They were coloured intensely red by Giemsa's stain.

On the 17*th day* a change was detected. A small minority of the parasites were elongated (Fig. 43) and some dumb-bell forms were seen (Fig. 44) indicating the possibility that division occurred though we could not prove that it did.

On the 18*th day* the alteration in morphology was more marked. Small forms with tail-like cytoplasmic processes were observed (Figs. 45 and 47) and a few plump parasites made their appearance (Fig. 46).

Beginning with the 19*th day* and continuing to the 22nd, the differentiation was paralleled by degeneration. A proportion of the parasites, which we did not measure quantitatively owing to the difficulty of drawing a sharp line of division between active and disintegrating forms, lost their characteristic staining reactions (Fig. 50) and seemed to be on the road to destruction. Two stages, not previously seen, were discovered on the 19th day, namely the **V** form (Fig. 49) and a small triangular parasite (Fig. 54). The latter was, however, of very rare occurrence. The tailed parasites (Fig. 55) had grown and the plump forms (Figs. 52 and 53) were larger and more numerous.

On the 20*th day* the differentiation of tailed (Fig. 60) and plump parasites had progressed somewhat, unmistakable signs of degeneration were also present (Figs. 59 and 62) and the first "cross" form was discovered (Fig. 57).

During the 21*st and* 22*nd days* the alterations were not very pronounced (see Figs. 63–69 and 70–76). The tailed and plump forms increased slightly in frequency though both were outnumbered by the small spherical or ovoid parasites observed continuously in the erythrocytes from the 16th day.

Counts were then made of the number of the various morphological forms of the parasite at a definite time in the blood of twelve animals in order to ascertain whether any numerical relation exists between them. The results are presented in Table VIII which for the most part explains itself. It is important, however, to define clearly the classification of parasites employed.

(1) *Spherical-ovoid* forms are the smallest parasites seen. They are indistinguishable from single gamonts. Like the gamonts their nuclei are roughly spherical and stain more intensely red by Giemsa's method than the nuclei of other developmental stages of the parasite. They correspond in part to the "round or ovoid" parasites of Nuttall and Fantham (1910); but we exclude from our group, which we admit is an artificial one, ovoid parasites whose cytoplasm equals or exceeds their nuclear volume. These spherical-ovoid forms are illustrated in Figs. 42, 72 and 93.

(2) *Rod-like* parasites are like the spherical-ovoid ones except that their length is approximately twice their diameter (see Fig. 43). Their diameter is approximately uniform throughout and their ends are rounded. They stain

intensely and are made up chiefly of nuclear material. Perhaps they are the same as the rod-shaped parasites of Nuttall and Fantham. They are definitely shorter than the bacilli-form parasites of these authors and much richer in chromatin.

(3) *Dumb-bell* parasites (Figs. 44 and 70) contain chromatin staining almost as intensely as that of the parasites of the two preceding categories. Like them, also, they possess very little cytoplasm. Under this heading we include couples of single spherical parasites when the two individuals are of the same size and in close apposition to each other. It is not necessary that they be joined by a strand of material representing the part of the dumb-bell grasped by the hand.

(4) **V** *parasites* are of such striking appearance that space will not be taken to describe them. They are illustrated in Nuttall and Fantham's Figs. 17, 19 and 20 and in our Figs. 49 and 66.

Table VIII. *Forms of parasites observed in red blood cells.*

Number of animal	4810	5723	5740	5759	5760	5774	5840	6673	7539	7600	7613	7644
Number of reds examined	205	162	248	310	238	493	557	662	141	254	296	229
Number containing parasites	101	139	142	69	137	59	51	302	131	54	266	56
Number of spherical-ovoid parasites	—	—	—	7	—	—	—	58	83	—	55	—
	11	77	16	1*	20	7	16	2*	3*	7	2*	11
Rod-like parasites	6	5	0	1	4	3	1	8	2	0	2	0
Dumb-bell parasites	0	10	7	2	4	0	2	7	19	3	2	2
V-shaped parasites	1	1	2	1	2	0	1	0	13	0	2	0
"Cross" forms	1	0	3	0	0	0	0	0	0	1	0	0
Tailed parasites	30	35	75	41	69	24	17	102	69	13	129	17
	9*	1*	2*	5*	3*	5*	1*	6*	5*	2*	6*	
Plump parasites	63	53	79	19	54	31	9	146	71	34	97	29
	16*	15*	7*	3*	6*	2*	3*	11*	8*	2*	11*	5*
Number parasites examined	137	197	191	78	162	75	60	335	273	62	306	64
Average number parasites per cell	1·2	1·4	1·3	1·1	1·1	1·2	1·1	1·0	2·0	1·1	1·1	1·1
Maximum number parasites per cell	4	7	4	3	5	3	2	4	8	3	3	3

* Forms exhibiting evidence of degeneration.

(5) *Cross forms* (Figs. 57, 63 and 85) are listed separately because they are prominently referred to in the literature.

(6) *Tailed parasites* constitute a large and important group. As we define them they are long slender parasites whose cytoplasm never exceeds their nucleus in diameter. Often the cytoplasm tapers to a point as in the comma-shaped and piriform parasites of various authors; but sometimes it is fairly uniform in thickness throughout its length. In the latter case they are somewhat bacilliform in appearance. The nucleus is usually at one extremity, the tail stretching away from it. The nucleus stains less intensely than the nuclei of the parasites of groups 1, 2 and 3. Tailed parasites are represented in Figs. 47, 55, 84 and 92. The variations in size can be estimated by comparing Figs. 47 and 84. The condition illustrated in Fig. 79 was taken as indicating the presence of five-tailed forms.

(7) *Plump parasites* are also very numerous. In general they exhibit a

greater amount of cytoplasm than of nucleus. Volumetrically they comprise the largest parasites noted. Though the nucleus is sometimes spherical it is more often flattened or cup-shaped with the concavity directed toward the cytoplasmic mass. It is frequently the palest staining of all the different forms of nuclei. The cytoplasm also is usually feebly coloured, especially in the concavity of the nucleus which has a washed-out look. Such parasites are shown in Figs. 46, 53, 65 and several others. They range in size from small ones (Fig. 71) to others which are much larger (Figs. 69 and 87).

Since gradations exist between these stages the distinction is one of degree only. The classification is obviously arbitrary, for specific mention is not made of parasites possessed of several masses of chromatin and of the rarer types. These and all the other counts reported in this paper were, however, made by one of us (E. V. C.), so that there is an element of uniformity about them which could not have been obtained had the counts been made by two different individuals. An analysis of the figures brings to light several interesting facts though the total number of parasites classified was only 1940:

(1) The degeneration of parasites within the red cells already described in calf No. 5840 (Figs. 50, 51, 59 and 62) occurs in varying degrees in all of the animals. The parasites in question often, while retaining their distinctive morphology, lose their characteristic tinctorial properties. Both chromatin and cytoplasm stain less intensely than those of neighbouring parasites, sometimes even within the same cell. They seem to fade away. Others break up so that irregular masses of cytoplasm without nuclei are observed, as well as nuclei with cytoplasm. In tailed parasites the tails may become separated from the nuclei. The nuclei themselves occasionally go to pieces before the cytoplasm shows marked signs of degeneration. The degeneration of parasites in the Koch body stage has been referred to. This recognition that degeneration accompanies growth and differentiation during the time that the parasites inhabit red blood cells paves the way for a more accurate interpretation of the life cycle. It leads us to scrutinise atypical parasites containing unusual looking nuclei with special care, particularly when the latter are of unequal size, and to hesitate before accepting all of them as stages of differentiation. We have no means of knowing how rapidly the process of degeneration can take place and result in the disappearance of parasites, but that some are removed in this way is clear. It follows that a given red cell may during the course of the disease reaction possess more parasites than was formerly supposed or can be seen at any one time.

(2) The three commonest stages can be classified as spherical-ovoid, tailed and plump forms. Taking totals their observed ratios were 379 : 664 : 782. They consequently serve as bases between which one would naturally search for transitions.

(3) The four other types are in all cases less numerous. They are the rod-like, dumb-bell, V and cross forms, with total ratios of 32 : 58 : 23 : 5. In their case degeneration is so conspicuous by its absence that it is a fair

assumption, backed up also by their small number, that the existence in time of these forms is relatively short as compared with the others.

(4) In making the table it was observed, though to avoid complications it is not recorded, that parasites of the same morphological type vary considerably in size as studied in the red cells of different animals. Thus in No. 5740 the tailed forms are three or four times as long as in No. 7539, and the plump forms are correspondingly larger. This may be due to an influence of the host on the parasite. At any rate it shows how essential it is to examine the blood of many animals before reaching conclusions.

We shall now proceed with the aid of Diagram I to a highly theoretical formulation of the changes which may take place in the intra-erythrocytic growth and differentiation of the parasites. In the first row of diagrams the possible stages in division of the spherical-ovoid form of parasite are depicted. It presumably increases in size (*b*), becomes gradually elongated and gives rise to a rod-shaped parasite (*c*). This becomes equatorially constricted and gradually segments (*d* and *e*). The two daughter parasites remain for a time together and later separate (*f* and *g*).

In the second row is represented the possible growth and division of a parasite which looks at first the same as a spherical-ovoid form but which acquires more and more cytoplasm. This modifies the process of division so that longitudinal fission takes place instead of transverse segmentation. Figs. 91 and 94 are more detailed drawings of stages *e* and *f*. Figs. 81 and 79 suggest: (1) that the single nucleus (*d*) may split into more than two parts, (2) that two or more parasites closely applied together may divide almost simultaneously, or (3) that having been produced by division the daughter parasites tend to adhere to one another by their cytoplasmic processes which may be more sticky. Significance of some kind is lent to conditions like those illustrated in Figs. 79, 81 and 89 because they are the exception rather than the rule. From the physical point of view one would expect the parasites to clump together like bacteria much more firmly than they do. It almost seems as if they repel one another, carrying perhaps the same kind of electrical charge. The resulting forms have much more cytoplasm than those represented in the first row. In the absence of evidence we assume that these may differentiate in two directions, indicated by the diagrams in rows *3* and *5*, developing on the one hand into typical tailed forms and on the other into plump forms.

The possibility should be entertained that the long-tailed form may itself divide (row *4*) because such parasites occasionally possess two and even more masses of chromatin. In the living state we have seen long-tailed forms partly segment so that they appeared to be on the point of developing into two parasites, but the thread of substance connecting the two parts persisted and actual division was not seen.

Appearances pointing to the possible division of plump forms are illustrated in the last row of diagrams. Parasites like all the stages represented are frequently met with (see Fig. 87).

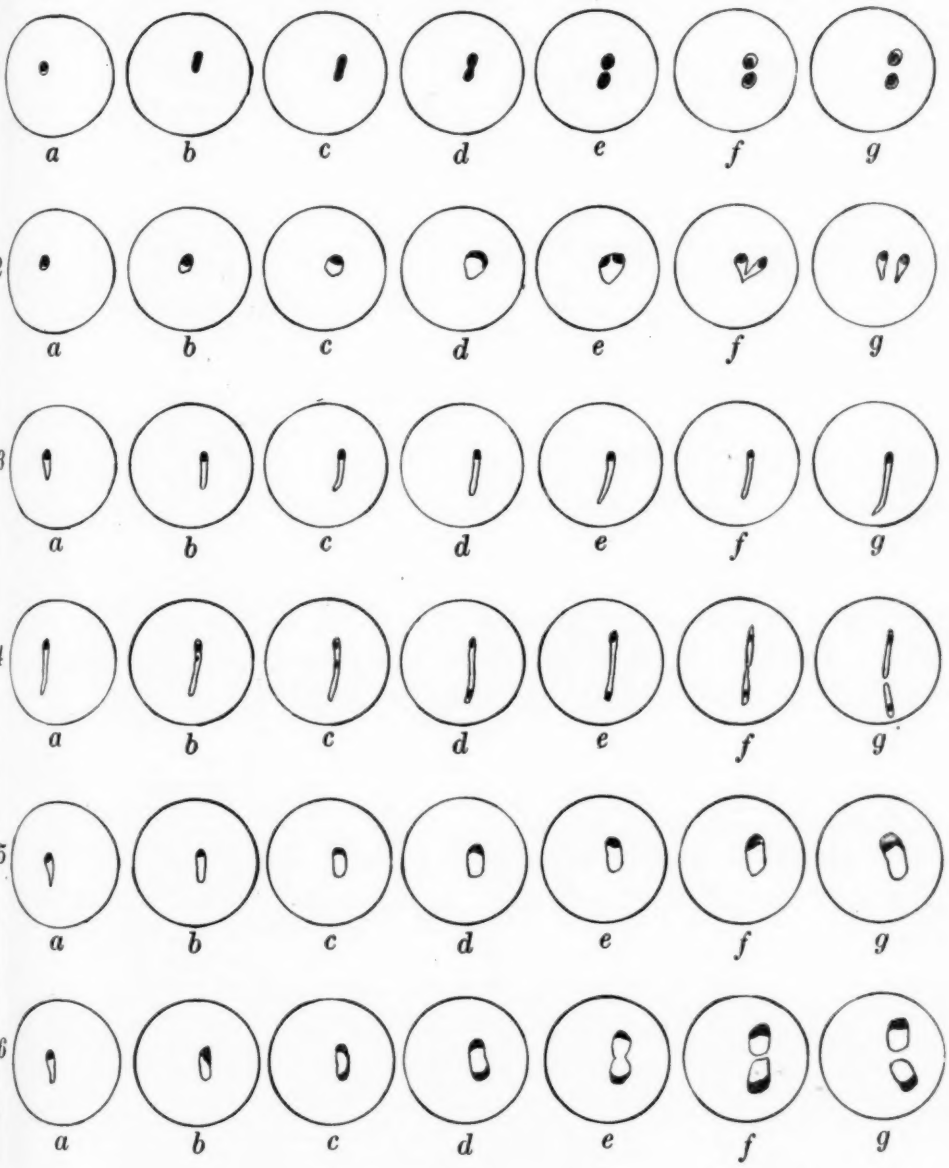


Diagram I. Illustrating possible stages of division of different intra-corpuscular forms of *T. parva* (*1–6*) seen in stained blood films. Refer to text for description.

We agree with Nuttall (1913) that the evidence that division occurs is not satisfactory, yet we think that multiplication takes place perhaps in one or other or all four of the ways which we have just outlined. It will be observed that our plump forms resemble Gonder's (1911 *a*) macrogametocytes and that our tailed parasites look like his microgametocytes, but we are not ready to assign these names to them. Further and more detailed studies will be required before the status of these intra-erythrocytic parasites can be understood.

V. Discussion.

(1) *Life cycle of* Theileria parva *in cattle*[1].

The principal changes are illustrated in Diagram II, which is made up of four columns indicating (*a*) the infective stages transmitted to cattle by ticks, (*b*) the agamogenous generation, (*c*) the gamogenous generation, and (*d*) development of the parasite in red blood cells.

(*a*) *Infective stages.* The act of feeding induces the development of infective stages (sporozoites) from the sporoblasts in the cells of the salivary glands (see Part I). These are passed into the host by the ticks sometime between the 3rd and 5th day after attachment of the ticks. Since the same salivary gland cells at this time also contain sporoblasts it is possible that the tick transmits some of the sporoblasts also, but we have no evidence that this is the case. We were not successful in detecting sporozoites in the corium at the site of attachment. The sporozoites, numbered (*1*) in the figure, will presumably all degenerate (*D*) if the animal is not susceptible, and some may degenerate even though it is. Others will pass in lymphatic channels to the nearest lymph glands where they enter principally the medium-sized lymphocytes (*2*) and give rise to agamonts. Lymphoblasts, small and large lymphocytes are also parasitised but to a less extent. It is conceivable also that the parasites may invade the lymphocytes at the site of the bite and travel within them to the nearest lymph glands.

(*b*) *The agamogenous generation.* Agamonts were first seen in lymph-gland smears the day the temperature first exceeded 103° F. Details of observations on several animals have been given in Tables II and VI. Meyer found them the second day after increase in temperature, but in no case, as far as he was able to ascertain, were they present on the day when the temperature increased.

Within the lymphocyte the parasite grows and becomes multinucleated,

[1] In the diagram of the life cycle of *T. parva* in the tick given in Part I (*Parasitology*, **24**, 32), the direction of the arrow on the upper right-hand side should be reversed.

Explanation of Diagram II.

Diagram II. Illustrating the life-cycle of *T. parva* (*a*) sporozoites in salivary glands of tick (*Rhipicephalus appendiculatus*), (*b*) agamonts, (*c*) gamonts, (*d*) intra-corpuscular parasites. (*b*), (*c*) and (*d*) occur in infected bovine and may be inbibed by the tick in blood sucked from infected host as indicated by arrows leading down from stages *3–5*, *7–11* to numbers *I–VII*. (*D*) indicates forms that may degenerate. Refer to text for description.

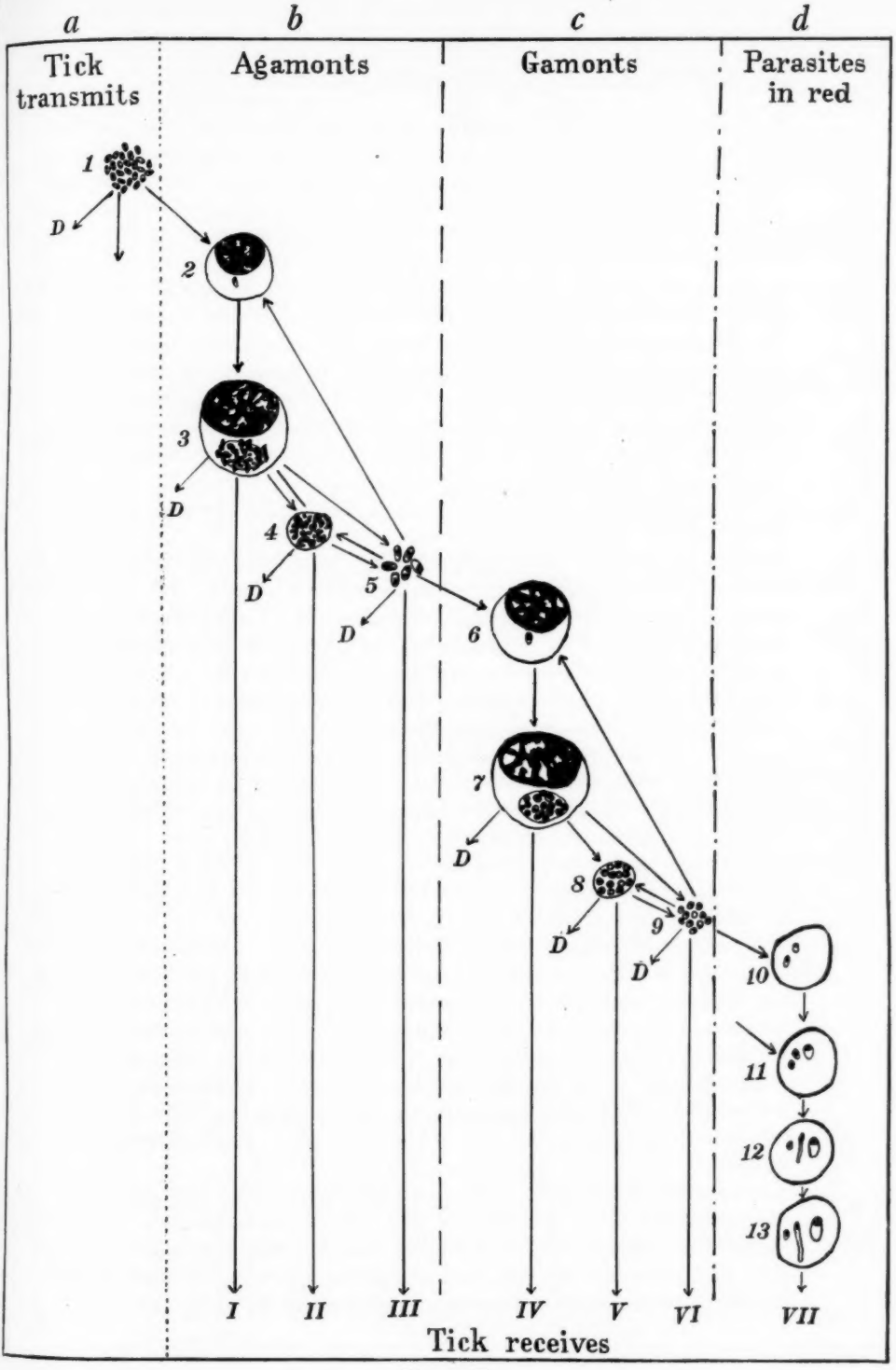

a
b
c
d
Tick transmits
Agamonts
Gamonts
Parasites in red
1
D
2
3
D
4
D
5
D
6
7
D
8
D
9
D
10
11
12
13
I
II
III
IV
V
VI
VII
Tick receives

Diagram II.

developing into a typical intracellular agamogenous Koch body (*3*). In order to avoid complicating the diagram we have not illustrated lymphocytes containing more than one Koch body. Two and even three are fairly common, while larger numbers are rare. Often they are of different sizes, pointing perhaps to successive penetration by individual sporozoites, some of which have had longer to develop than others.

The lymphocyte containing the Koch body may degenerate (*D*) or find its way into the blood (*I*), or the Koch body may escape from it and live a free existence in the fluid of the lymph gland (*4*). Such extracellular agamogenous Koch bodies have three possible fates indicated from left to right in the diagram. They may degenerate (*D*), or persist and be washed into the circulation (*II*), or they may break up into a swarm of individual agamonts (*5*).

Individual agamonts (*5*) are probably more frequently formed by an immediate partition of an intracellular Koch body (*3*) coincident with the disintegration of the cell containing it. The length of life of the parasite as a free agamont is not known. Many may degenerate (*D*) depending upon the resistance of the animal. Others may endure and pass into the circulation (*III*), or through division give rise to free extracellular Koch bodies (*4*), or infect other lymphocytes (*2*) developing intracellularly into Koch bodies (*3*) either in the lymph glands or in the blood stream and repeat the agamogenous cycle resulting in rapid and wholesale multiplication of parasites, while still others may invade different lymphocytes and give rise to the gamogenous generation (*6*).

The time required for the differentiation of agamonts capable of forming gamonts is not accurately known. But in calf No. 5840 (Table II) the agamogenous Koch bodies preceded those of the gamogenous variety by only 2 days. There is reason to believe that the time may be shorter as well as longer (calf No. 5869), because the first parasites were sometimes seen in red cells (after the gamogenous generation) the same day that the first Koch bodies were observed in lymph-gland smears, though they may have been present unnoticed in the latter beforehand.

(*c*) *The gamogenous generation.* This generation, like the preceding, may develop from single (*6*) or multiple (not represented) infection of lymphocytes. The Koch bodies (*7*) are, as Gonder discovered, quite different in character from the agamogenous ones. Their individual nuclei are smaller, more nearly spherical and possess a far greater affinity for stains than those of the agamonts (Figs. 10, 11, 25–29, 33–36). The cytoplasmic background of the entire gamogenous Koch body has less affinity for the blue component of Giemsa's stain, and in the individual gamont is of smaller extent than in the agamonts (see Figs. 11, 30–32 and 38–40).

At first the history of the intracellular gamogenous Koch body is like that of the agamont. It may degenerate (*D*), enter the circulation (*IV*), escape from the lymphocyte (*8*), or break up with the lymphocyte into single gamonts (*9*). In case it persists as a composite body (*8*) it may likewise gain the blood stream (*V*) or break up into single gamonts (*9*)

The single gamonts either degenerate (*D*), persist in the blood (*VI*), develop extracellularly into other Koch bodies (*8*), infect other lymphocytes (*6*) and repeat the cycle just as the agamont does, or enter the circulation and invade red blood corpuscles (*10*). Since neither the lymphocytes nor the parasites can be marked it is impossible to follow the behaviour of any individual. It is for this reason that we cannot do more than suggest possibilities. How often the agamogenous and the gamogenous cycles are repeated remains an open question. The time necessary for the gamonts to mature cannot be longer than that required for the agamonts. On the average, parasites were first observed in the red cells 1·6 days after the first Koch bodies were seen (Table VI). It would appear, therefore, that the development of both agamonts and gamonts is in some cases only a matter of hours.

Further studies on agamonts and gamonts are needed. At present we do not know which is the most dangerous to the host. Neither have we any data at all bearing on factors which facilitate or retard the change of agamonts into gamonts. Much may hinge on the discovery of what they are. If the change can be in some way delayed or prevented it is possible that the animals would survive. One practical difficulty already mentioned is that the behaviour of individual lymphocytes carrying Koch bodies or of groups of them cannot be followed under the complex conditions which obtain in the living animal. It is possible that this might be partially surmounted by the use of special methods of tissue culture. An attempt to adapt the technique for the preparation of cultures of lymphocytes (Carrel and Ebeling, 1922; Maximow, 1928) to the culture of bovine lymphocytes is indicated.

(*d*) *The parasites in red cells.* They were first observed (*10*) between 9 and 26 days after infestation with ticks, the average being 16·4 days (Table VI). They appeared between 2 and 17 days after the temperature surpassed 103° F., the average being 6·6 days. Based on the time of maximum temperature they were first seen between 4 days before and 7 days after, with an average of 0·6 day after. Their gamogenous origin is clearly shown by their small size, intense staining reactions and the small amount of cytoplasm. It is likely that all the parasites within a red cell have not entered at any one time, but at intervals over a period of undetermined length. Thus, the appearance of an additional parasite is represented in stage *11*. With development there is an alteration in morphology (*10–13*). The small spherical-ovoid forms which dominated the picture at the beginning give place as far as numbers are concerned to tailed and plump forms. Possible stages in the growth and multiplication are illustrated in Diagram I and have already been fully discussed. The fact that degeneration of some parasites accompanies the differentiation of others suggests (1) that forms of irregular morphology and tinctorial properties do not necessarily belong to the developmental cycle, and (2) that with the loss of some parasites multiplication may take place more regularly than is supposed and without apparent crowding of the cells with parasites.

The life cycle of the parasite in the vertebrate host terminates at the time

of the death of the host, with the possibility of the coexistence in the blood of the seven stages of the parasite indicated at the bottom of Diagram II. The dominating parasites, as far as numbers are concerned, are those in the red blood cells and the gamonts. We have shown in Part I that it is the former which are capable of initiating the cycle in the vector if ingested by ticks of the appropriate species; but we have not succeeded in definitely identifying gametes or gametocytes or in distinguishing between the sexes. This problem will doubtless be attacked by others. We feel (1) that a careful comparison made in smears of forms seen in the blood of the vertebrate host with those observed in the blood soon after its ingestion by the vector might prove fruitful by revealing stages in the latter not present in the former, and (2) that a comparison with *T. parva* in the blood of the host with other species of the genus, which by contrast are readily transmissible by blood inoculation, might bring to light in the latter forms which really are gametocytes or gametes.

(2) *Reaction of the tissues to the parasites.*

Evidence on which to base a comprehensive account of the tissue reactions to the parasites is lacking in the papers of our predecessors as well as in this contribution. For reasons already stated we failed to find sporozoites in the corium at the sites of attachment of transmitting ticks. It is only by discovering how the cells in this locality behave with reference to the sporozoites that data can be secured on the nature and mode of action of this, the first line of defence. The mechanism of the response should be analysed by comparative studies on (1) susceptible animals, (2) recovered and at the time relatively immune animals, and (3) animals of a species which, though exposed, never contract East Coast fever.

Except for our series of excised lymph glands and the tissues of a few calves and immunes we have practically confined our observations to the lesions exhibited by animals killed *in extremis*. As far as we have been able to ascertain the literature does not contain a systematic account of the development of the lesions in the various organs and tissues. This is much needed because until such information becomes available some conditions which antedate the East Coast fever reaction may be confused with it. For example, the endarteritis obliterans observed by Collaud (1906) in the kidneys of animals which died of East Coast fever may not in any sense represent a direct or indirect tissue response to the parasites. Since it is not an acute, rapidly developing condition, whereas East Coast fever usually is, it would seem likely that the endarteritis was present unnoticed before the East Coast fever reaction began. The causal relation between East Coast fever and ulcers in the stomach reported in the literature is likewise questionable. The "white foci" in the kidneys, which have been considered almost pathognomonic of the disease are sometimes absent or at least not noticeable macroscopically in typical cases. We naturally ask ourselves whether it is always simply a question of

the more rapid development of these foci in some animals or whether we have to do with the accentuation (perhaps at different rates) of lymphocytic infiltrations already present, under the influence direct or indirect of the parasites? In favour of the latter possibility is the fact that perivascular lymphocytic infiltrations, which are sometimes sharply localised and seem to be focal, are now recognised as of frequent occurrence in the domestic animals and man entirely apart from East Coast fever. In the kidneys of rabbits, for instance, they may result from encephalitozoan infections, not only in one but in many parts of the world (Cowdry, 1926), which are so mild as to be unrevealed by any clinical symptoms. They are the kind of lesions known to be brought about by a wide variety of causes.

It is essential to replace vague statements that lesions are "slight," "marked," "severe" and so on, in which the personal equation of the observer means everything, by quantitative measurements. Only in respect to the blood and the body temperature does the literature contain quantitative data bearing on the reaction of the host to the parasite. We have made some progress by determining the number of cells per c.mm. in the lymph glands and perivascular infiltrations, the number of which are dividing, contain Koch bodies, etc. (Tables IV and V); but much remains to be done.

Though our information is fragmentary concerning the response of the tissues in East Coast fever, some features of it are plain:

(1) The medium-sized lymphocytes, first in the lymph glands and later in all parts of the body, are the chief cells infested. The parasite multiplies within them without quickly killing them. Our observations do not support the current view that the parasite acts as a powerful stimulus to the multiplication of lymphocytes. The swelling of lymph glands is due more to oedema than to increase in number of lymphocytes. In the circulating blood Steck has found that both lymphocytes and leucocytes steadily decrease in number as the disease progresses. It is true, as he observed, that the lymphocytes stagnate in large numbers in the vessels of certain organs, notably the liver, but it is difficult to prove an absolute increase in the body as a whole. Perivascular lymphocytic infiltrations are sometimes quite marked, but not more so than in several human diseases, or in Snotziekte, judging from Mettam's (1924) description and from preparations made by one of us (E. V. C.) while in South Africa.

(2) Small haemorrhages, noticeable at autopsy, are often but not invariably a conspicuous feature of the disease, particularly in the endocardium and epicardium. Haemorrhages somewhere in the body were seen by Sir Arnold Theiler in fifteen out of sixteen cases, by Steck in nine out of fourteen and by us in eight out of ten. Owing to the circumstance, already cited, that the terminal lesions have alone been consistently investigated, we do not know at what time these haemorrhages occur with reference either to the febrile reaction or to the phases in development of the parasites or to changes in their number. We have not been able to confirm K. F. Meyer's contention

that these haemorrhages offer pathways by which the parasitised lymphocytes assume a perivascular position. It is interesting to note, however, that in describing the evolution of the perivascular infiltrations in Snotziekte, Mettam makes the following remark: "The initial stage in the development of the lesion is a stasis of the blood in the vessels of the part, which perseveres throughout the disease. This is at first followed by the appearance of some erythrocytes outside the blood vessels and very shortly followed by an increase in the number of small lymphocytes in the immediate vicinity of the vessels...." He does not refer specifically to haemorrhage. The stasis may be an important factor in East Coast fever as well as in Snotziekte.

(3) Oedema was observed in all of the lymph glands which we examined after the disease had been definitely established. It is also mentioned in the literature. In the lungs it is relatively common. Theiler reported it in twelve out of sixteen cases, Steck in seven out of fourteen and we found it in three out of ten. This, like the lymphocytic reactions and haemorrhage, is an acute process.

(4) Though in East Coast fever the animals lose weight and their general condition becomes poor, indicating that physiological processes are seriously interfered with, the injury is not of sufficient standing to give rise to distinctive structural changes in addition to those which have been mentioned. Despite the fact that almost all of the erythrocytes may have been invaded by parasites there is little if any anaemia (Strickland, 1915). We are not in a position to say which vital activity is so impaired that death results. This is an acknowledgment of failure on our part and on the part of those investigating the disease for more than a quarter of a century.

Our information is superficial and leaves out of account what is probably the key to the problem, namely the disturbance of lymphocytic function resulting from the direct action of the parasites. This must be regarded from both qualitative and quantitative points of view. We must discover the kind of disturbance as well as its extent.

Further studies, like those reported in this paper, may be expected to answer finally the question as to whether the parasites act as a stimulus to the multiplication of lymphocytes. The technique devised by Barron and Harrop (1929) is adequate for the measurement of glycolysis, respiratory power, the Pasteur reaction and similar attributes of lymphocytes. Parallel cytological studies on motility, oxydase and peroxidase reactions, response to supravital dyes and the like should be made. It would be surprising if such an analysis did not reveal physiological modifications of some kind in the parasitised lymphocytes. Though the reason for the death of the animal as a whole eludes us, it should be possible to discover why the lymphocytes die.

On the quantitative side it is important to correlate and balance (1) the diminution in lymphocytes in the peripheral blood and their concentration in the vessels of the liver and other organs noted by Steck, (2) the decrease in lymphocytes in the lymph glands which we emphasise, and (3) the increase

in perivascular lymphocytes which constitute the infiltrations. This means the testing of lymphocytes in these situations by the qualitative methods described which have proved the most useful and the making of quantitative studies on the relative numbers of (*a*) lymphocytes + Koch bodies, (*b*) lymphocytes – Koch bodies, (*c*) dividing lymphocytes + Koch bodies, (*d*) dividing lymphocytes – Koch bodies, (*e*) degenerating lymphocytes + Koch bodies, and (*f*) degenerating lymphocytes – Koch bodies. Normally the duration of the life of lymphocytes in the circulating blood is very short. It is known that more enter the blood stream in 24 hours than are present in it at any one time; but in East Coast fever the local intravascular stagnation of lymphocytes introduces a complicating factor. It is safe to assume that this is due to changes in the lymphocytes themselves, to changes in the circulatory mechanism, or to both. Application of the qualitative tests might be helpful. Barron and Harrop have observed that when lymphocytes are overcrowded they lose their ability to respire more rapidly than the polymorphonuclear leucocytes do. An analysis of the process of stagnation may explain also the emigration of lymphocytes as a factor in the development of the perivascular infiltrations.

The particular interpretation of the results which any investigator may offer in an attempt to explain why animals die of East Coast fever will largely depend upon his conception of the nature of lymphocytic activity. Until more data are secured all that can be said is that it will be conditioned by (1) the possibility of the liberation of a poisonous substance by the parasites, (2) the influence of the products of the degeneration of lymphocytes, and (3) the physiological loss to the organism brought about by the destruction of billions of lymphocytes and the as yet hypothetical impairment of the activity of a large proportion of the remaining ones.

While there is no basis in fact for the consideration of a poisonous substance or toxin, the febrile reaction must be caused by some material circulating in the blood which readily diffuses through the walls of vessels. It is doubtful whether we have to do simply with some product of lymphocytic degeneration because of lack of close coincidence in time. In one case the temperature surpassed 103° F. the day the parasites were first noted in the lymphocytes at a time when they could have produced but little degeneration. The temperature, moreover, usually subsides quickly in later stages when degeneration reaches a maximum. But the products of the degeneration of lymphocytes may well be responsible for the oedema and the haemorrhages which by contrast to the febrile reaction are delayed manifestations of the disease. The case for oedema is better than that for haemorrhage for the reason that oedema always occurs, especially in the lymph glands where much degeneration is localised, whereas haemorrhages are occasionally looked for in vain in otherwise characteristic cases of East Coast fever. It is not improbable that the products of degeneration may exercise an inhibiting effect on physiological functions, which we cannot now identify, and thus promote

the wasting and loss of condition exhibited by moribund animals. Evidently these products may be different in kind from those regularly set free by the disintegration of lymphocytes in normal animals. Another possibility is that the wasting may be occasioned partly or wholly by the progressive failure of more and more lymphocytes to perform their proper functions. Maximow (1930) has stated that "it is usually supposed that their function is connected with their quantity in the circulation." If Mottram, Cramer and Drew (1922) are correct in their belief that their studies on radiation confirm Schafer's contention that the lymphocytes play an important part in fat metabolism, then injury to them may actually be the direct cause of the loss of condition mentioned.

(3) *Mechanism of recovery.*

The tissue reactions leading to recovery remain a closed book. Like Steck, we hoped to investigate them, but were unable to do so on account of the perfectly understandable difficulty of securing material. We have had only two animals (bovines No. 5922 and 6927) in neither of which could we detect the persistence of Koch bodies or any point of interest. It did not seem desirable to delay further this publication until other specimens were sent to us by the kindness of officials in Kenya. A large series of animals would be necessary, killed at intervals after the subsidence of acute symptoms. The observation of Bruce (1910) that the lymph glands of calves recovered from Amakebe (probably East Coast fever) remain permanently enlarged suggests the desirability of a correlated clinical and histological study of the process of recovery in which the enlarged glands would be measured at intervals and excised for histological examination by the methods which we have employed.

Whether cattle apparently recovered from East Coast fever still harbour the parasites is a question demanding answer. If the parasites do persist and the proper ticks are present as vectors, such cattle are possible sources of danger to susceptible animals, in other words, they are "carriers." Gonder and all others after him have failed to find parasites in immune animals. The search should be intensified and new methods employed because of the possibility mentioned by Bevan (1924) that the parasites "...may be present in some unrecognised form which may remain latent or dormant until such time as the influence which restrains them is relaxed and their development can recommence." On the basis of his observations published in 1924 (we have not been able to consult his earlier contributions) we are not, however, ready to accept his conclusion that relapses occur, because it seems to us that he has not altogether excluded the possibility that reinfection may have taken place.

Probably realising from experience with other Hematozoa that passive immunity is in some cases coextensive with the continuation of the infection in a latent unrecognisable form, Du Toit (1931) very properly attempted to

produce a relapse in seemingly recovered animals, but without success, though he employed splenectomy as advised by de Kock and Quinlan (1926) and other likely methods. Theoretically, however, a clinically noticeable relapse may not be prerequisite for ticks to ingest parasites from passively immune animals. Though they may not be detectable microscopically owing to their small numbers, except by a happy accident, a few may still occur and lead to transmission as in bird malaria. Whether they do remain or not is the question.

(4) *Prevention.*

If it is found that passive immunity only remains as long as the parasites do, the chances for creating a strong, persistent, active immunity are not favourable. Almost since the discovery of East Coast fever attention has been focussed upon this problem of immunisation. The literature can be secured from the bibliography of Schilling and Meyer (1930). Among the more recent papers, those of Walker (1930), Walker and Whitworth (1929), and Du Toit (1931) may be mentioned. A measure of success has been obtained, but the results are inconstant and there is an element of danger for the inocula contain living parasites. If by some therapeutic means the resistance of the animals could be temporarily raised the chances of contracting a fatal attack of East Coast fever would be reduced. Subsequent inoculations at regular periods might "hold" the immunity.

The principal obstacle in the way of progress in this direction is that experiments must be confined to large and expensive animals of one species. We are informed by Mr James Walker that the Indian water buffalo is also susceptible, but it likewise is far too large. The discovery of a small susceptible animal is highly desirable. The outlook at first sight would seem almost hopeless because of the well-known general host specificity of piroplasms. Nevertheless, there have been such important advances in transmitting various infective agents to animals which under natural conditions are never infected that it would appear worth while to make repeated attempts. Before the success of Max Theiler (1930) no one would have thought it possible that yellow fever could be transmitted to ordinary white mice or would have predicted the flood of light on the problem which has resulted. Even though yellow fever is an entirely different disease the methods employed, directed at the lymphatic system, are worthy of trial. Thus far in the attempts at artificial transmission of East Coast fever, by injection of blood, lymph gland and spleen emulsions and even by transplantation, to susceptible animals and to species which are apparently not susceptible, no effort has been made to reduce the local resistance of the receiving tissues or organs. Yet there is evidence that the resistance to other infective agents can be lowered experimentally. It is sufficient to cite the investigations of Flexner and Amoss (1917) on poliomyelitis, of Zwick, Seifried and Witte (1929) on Borna disease, and of Sawyer and Lloyd (1931) on yellow fever. How one would proceed to

depress the resistance of the lymphatic system to *T. parva* would be in itself a fascinating physiological problem.

The experiments of Goodpasture, Woodruff and Buddingh (1931) show that, though repeated attempts to infect adult and young chickens with the virus of herpes have failed, the much younger cells of the chorio-allantoic membrane of unhatched chicks are readily susceptible to it. It is a long step from the herpetic virus to *T. parva*, but the possibility that the resistance to it also is in some way conditioned by the age of the tissue should be explored by the same technique, using chicks as material.

Fortunately, prevention by avoidance is better understood than by immunisation. Our observations detailed in the first paper have confirmed the already satisfactory evidence that the parasite is not transmitted hereditarily from one generation to another, so that unless some unknown animal reservoir for the parasite exists it will not survive more than the life span of a single generation in the absence of susceptible animals. Theiler (1905) has contributed accurate data on how long an area remains infected with East Coast fever. Theiler, Gray and Power (1915), Cooper and Laws (1915), Knuth and du Toit (1921) and others have also discovered efficient means of dipping cattle with a view to the eradication of ticks. But tick surveys should be made by a skilled entomologist with the object of securing accurate information on: (1) the relative number of ticks of the transmitting species; (2) the percentage of them which contain *T. parva*, using the technique reported in our first paper or a modification of it which permits of making preparations more quickly; and (3) the physical features of the terrain which facilitate or render difficult measures for reducing the tick population.

It would appear that the disease has spread to the entire East Coast of Africa from an endemic focus near Dar-es-Salaam, where it was first noted by Koch (1897). In view of the general conclusions of Hegner (1926) regarding host-parasitic relation, the disease reaction is so acute and violent that the parasite may be presumed to have invaded bovines relatively recently. East Coast fever in bovines at Dar-es-Salaam, as a matter of fact, probably dates from soon after the bovines were first imported, for they are not native to East Africa. It is logical to assume the pre-existence and continued existence of the parasite in some mammal in which it produces few if any symptoms. Already parasites resembling *T. parva* have been reported by Ross (1911) in a hartebeest (*Budalus cokei*), by Lichtenheld (1911) in an eland, by França (1913) in Grant's gazelle (in specimens collected by Stordy and sent to him by Montgomery) and by Montgomery (1913) in a topi (*Damaliscus corrigum jimela*). Further investigation is indicated of these and other wild animals. Animals, particularly in the vicinity of Dar-es-Salaam, on which ticks of species capable of transmitting *T. parva* habitually feed and which zoologically are nearest related to the bovines would first come under suspicion. The direct examination of the blood and tissues of such animals under normal conditions might and probably would be fruitless. But the number of parasites might

possibly be increased to the point of detection by splenectomy and other procedures of decreasing the resistance of the host. Perhaps the best method would be to try to repeat the transfer, which must have taken place naturally, by feeding clean ticks of the proper species on such animals, allowing them to moult and placing them on susceptible calves. A histological search for parasites in ticks collected in an area free from East Coast fever in bovines, if successful would be positive proof of the existence of a reservoir of parasites in some other kind of animal.

(5) *Therapy.*

Even now some basis exists for experimental treatments[1]. The high temperature indicates the administration of almost overwhelming doses of antipyretics because at first we must strive for maximum effects although an element of risk may be incurred. The influence of cold on the ticks and the parasite has been investigated by Theiler (1908), and by Nuttall and Hindle (1913). It is conceivable that the disease reaction could be arrested if freezing mixtures were applied to the superficial lymph glands which are first affected or if the animal were kept in a cold room. And, conversely, because the rise in temperature may turn out to be a physiological defence of importance, the temperature of the body and of the superficial lymph glands should be artificially raised at an early stage in the reaction. The involvement of the lymphocytes, the prevalence of small haemorrhages and the oedema also suggest lines of experimentation.

One would naturally try to influence the lymphocytes in every conceivable way on the chance that something helpful might be discovered. In a few animals we injected benzol, but without apparent result on the course of the disease. The effect of X-ray on the superficial lymph glands should be tested, though it is obvious that this procedure if found beneficial could only be used on animals of special value. In the Union of South Africa where Snotziekte exists it would be interesting to attempt to superpose the East Coast fever reaction on animals exhibiting this remarkable lymphocytic hyperplasia or *vice versa*. But unfortunately both are often so rapidly fatal that the time available might not be sufficient. The vulnerable stage of the parasite may be the change from agamonts to gamonts as already suggested, or the single agamonts and gamonts as they exist in the blood plasma and intercellular fluid of the lymph glands. The fact that the lymphocytes, or small round cells, of the thymus are not severely injured in East Coast fever, coupled with the observations that calves are sometimes more prone to recover than adults, would incline one to administer large amounts of thymus extract despite the observations of Perla and Marmorston-Gottesman (1930) that thymectomy has a favourable effect on the course of subsequent infection of rats with *Trypanosoma lewisi*. In view of the possible rôle of lymphocytes in fat metabolism it would be interesting to give large amounts of milk by the mouth,

[1] The clinical aspects of the disease are well described by Schilling and Meyer (1930).

intravenously and intraglandularly because this treatment might partly compensate the animal for the loss of lymphocytes in the course of the disease. In addition, the neutral fat taken in by the lymphocytes would alter their oxidation-reduction potential and perhaps influence the parasites indirectly.

The frequency of haemorrhages from small blood vessels points to the use of drugs which would improve the "tone" of the vessels. Energetic vaso-constrictors and vaso-dilators should also be tried, for the disease might thereby be in some way modified. It would be interesting to discover whether animals given ergot succumb more rapidly or develop more extensive peri-vascular infiltrations.

Since the oedema is probably of chemical rather than of nervous origin chemical means might well be applied to influence it, such as carefully graded injections of dilute acids and alkalis into the general circulation, into the superficial lymph glands, even into the lungs when there are clinical signs of oedema in them.

Exhaustive experiments have already been made in chemotherapy without avail. Nevertheless, work along this line should in our opinion be pushed even in a blind way because the influence of trypan blue in canine piroplasmosis and quinine in malaria could never have been predicted in advance. Our finding that schizogony is restricted to cells of the lymphocytic series and does not take place in endothelial cells explains the lack of utility of trypan-blue and similar colloidal dyes. What is needed is a substance which, even should it have no direct action on the parasite, nevertheless alters the permeability or some other physiological property of lymphocytes. We have the feeling that the literature on chemotherapy in man should be scanned for possible leads, for we find that many chemicals which have been used in man have, judging from published reports, not been applied to animals suffering from East Coast fever. For example, thorium dioxide regularly destroys lymphocytes, but sometimes exercises a very stimulating effect on the germ centres of lymph glands (Harris, 1932). There is a chance that this is exactly what is wanted, because if the elimination of the older medium-sized lymphocytes, which carry most of the parasites, could be linked with assistance to the animal in the production of more unaffected lymphocytes much might be accomplished. This interesting substance has the further property of being rather specifically adsorbed by lymph glands so that they are rendered opaque to X-ray. It might therefore serve to sensitise the lymph glands for X-ray treatment. There is no doubt but that it would facilitate X-ray photography of the glands. The experiments should, however, not be restricted to the use of rare and unusual preparations.

Some simple change in the circulating blood may affect the parasites profoundly. Reduction in the amount of blood sugar can presumably be quickly effected by insulin administration. The experiments of Hegner and MacDougall (1926) suggest that such reduction creates a condition unfavourable for the parasite of bird malaria. According to Taliaferro (1928): "In trypanosome

infections there is evidence that carbohydrates, such as sugars, are necessary for motility (which is probably a delicate measure of vitality) and that these disappear from the liver and blood as the infection progresses." The converse experiment of increasing the blood sugar should be made. Blood calcium plays quantitatively a particularly important rôle in the physiology of bovines. Indeed this is one of the attributes which it would be natural to investigate first as a possible factor in susceptibility to *T. parva*. Calcium in the blood can within the space of a few hours be increased by parathormone, and it can probably be precipitated out and thus materially reduced in amount in the course of a day or two by heavy doses of irradiated ergosterol (viosterol). The giving of large amounts of orthophosphate might also reduce calcium (Bulger, Dixon, Barr and Schregardus, 1930). This would be much cheaper than irradiated ergosterol. It is a simple matter to increase greatly the basal metabolic rate in the course of a few minutes by giving thyroxin.

Hegner's (1923) observation "that rats heavily infected with intestinal flagellates can be largely freed from their infection, especially with trichomonads, if fed for about a week" on a carnivorous diet together with the fact that a protein, peptone, is customarily added to emulsions of lymph glands or spleen used in East Coast fever for immunising purposes raises the question of the advisability of intravascular protein injections. That some animals might die of shock is beside the point. What is desirable is to shock the parasites by altering the environment in which they grow. In experiments such as these one should give doses to the limit of tolerance and reduce as seems desirable with the single object of making the blood stream for a short time as little like that of a normal bovine as possible with the intention of injuring the parasites, for as far as we know they can grow and multiply only with bovines (including Indian water buffaloes) as hosts.

A few preliminary experiments designed to reveal the behaviour and time of survival of active agamonts and gamonts under optimal environmental conditions *in vitro* and under carefully adjusted observation in the dark field might afford a basis for the rapid testing of the effects on them of foreign substances and of changes in amounts of materials to which they are regularly exposed. Such *in vitro* studies would be less reliable than those affecting the physiology of the host, but they might yield valuable clues. Tissue cultures might be helpful if the examination of small amounts of blood and lymph-gland juices mounted on ordinary slides over a few hours does not suffice.

As already noted there is a chance that at some time in the reaction between parasite and host a specific substance like a toxin may be produced. While this seems to be more the exception than the rule in Protozoa, such substances have been discovered. A case in point is the poison isolated by Pfeiffer (1891) from *Sarcocystis tenella*, for which Teichmann and Braun (1911) prepared a potent antitoxin. Schilling and Meyer (1930) speak of the toxic action of the agamogenous generation of *T. parva*. The chief difficulty, as Schilling (1930) notes, is experienced in isolating the parasites in sufficient

amounts. Perhaps this is not insurmountable. If it is, the testing of extracts of lymph glands, swarming with parasites during the course of the disease, for physiologically active substances ought to be entrusted to a pharmacologist. Should a substance of this kind be isolated and identified a rational basis would be afforded for treatment.

(6) *Systematic position of* T. parva.

Our observations lead us to question two properties which are often mentioned in definitions of *T. parva*.

The genus *Theileria* was established by Bettencourt, França and Borges (1907) to include: "bacilliform parasites which, in dividing, give cross forms. The elements of the cross are very small, rounded and almost exclusively made up of chromatin" (translated from França, 1913). We have illustrated these cross forms in Figs. 57, 63 and 85. On the basis of the study of stained preparations of *T. parva*, Nuttall and Fantham (1910) say that: "in certain parasites rich in chromatin, the mass may undergo division into four, giving rise to 'cross forms,'" and, in their description of figures they refer to: "Two rod-like parasites, lying across each other, forming an apparent 'cross form.'" We admit both of these possibilities and advance a third, namely, that cross forms appear when, through chance association, two dumb-bells develop in close apposition (Figs. 63 and 65). The fact that in the case of *T. parva* the cross forms are sometimes absent and never numerous (Table VIII), minimises their value as a diagnostic feature.

The second attribute is the kind of cell in which the parasite undergoes schizogony, which is incorporated in later definitions of the genus. We believe that it does not do so within endothelial cells as has been claimed by those who have accepted Meyer's conclusions. Thus Dennis (1932) states that "...the members of the family Theileriidae reproduce, also asexually, by a definite process of schizogony within the endothelial cells of the internal organs." Meyer's statement (1911) was perfectly clear, as have been his subsequent accounts. He evidently was convinced that the parasite formed Koch bodies within the typical endothelial cells lining the blood vessels. Du Toit (1918) has, however, used the term "endothelial" in a different sense. In his Fig. 9 *K* he has represented an "agamont in einer Endothelzelle"; but the cell, as illustrated, is roughly spherical and contains a rounded nucleus. The cell, moreover, is not flattened as vascular endothelial cells always are, neither are there signs of contact with the other endothelial cells which together make up the lining of a blood vessel; instead it has every appearance of being a cell which exists free in a fluid environment. In other words, it looks much more like some cell of the lymphocytic series. It is, indeed, in the cells of the lymphatic system that du Toit specifically says that schizogony takes place. Du Toit's use of the word "Endothelzelle" is therefore inconsistent with his general thesis. Wenyon (1926) has employed the designation "endothelial cell" rather more loosely. He states (1) in reference to *T. parva* that schizogony

occurs "in the internal organs within cells which are probably endothelial in nature" (p. 986), (2) in reference to the genus *Theileria* that schizogony takes place "in cells of the lymphatic system" (p. 991), and (3) in reference to the family Theileriidae that reproduction is "by schizogony within endothelial cells of the capillaries of the internal organs" (p. 1029). Later on he says that "occasionally they (the schizonts) are found in endothelial cells in the peripheral blood." There are those who agree with Mallory that "endothelial leucocytes" are simply altered and detached vascular endothelial cells, and du Toit and Wenyon may be among them; but the term "endothelial leucocyte" is not synonymous with the word "lymphocyte." We think that *T. parva* undergoes schizogony only in cells of the lymphocytic series, never in endothelial cells, and we consider it probable that other members of the group behave in the same way, notwithstanding the statement of Carpano (1915) that in the Mediterranean type of the disease it is the "cellule endothelial" which contains the parasites.

Wenyon suggests that "...the grouping of the piroplasmata in two families, Babesiidae and Theileriidae, corresponds with the subdivision of the pigmented *haemosporidia* into the two families, Plasmodiidae and Haemoproteidae. In the one the red cells contain asexually reproducing forms and gametocytes, while in the other the red cells contain only gametocytes, the schizonts occurring in the endothelial cells of the vessels." The analogy, however, only holds in a general way, for the Haemoproteidae are not easily transmissible by direct inoculation of blood, whereas some of the Theileriidae are (*T. dispar* and *T. mutans*) though *T. parva* is not. According to Brumpt (1924), *T. mutans* and *T. annulata* persist for a long time in the blood and there is no immunity, while *T. parva* and *T. dispar* soon disappear and there is immunity; but the evidence is not altogether satisfactory. Obviously comparative studies on these four species of Theileriidae in bovines are much needed along the lines of the excellent contribution of Sergent *et al.* (1927). Particularly should the relation between *T. parva* and *T. mutans* be cleared up (see Sergent, 1920; Theiler and Graf, 1928; Sergent *et al.* 1929; and Schwetz and Storck, 1930). It is not unlikely that differences in transmissibility are conditioned by the degree of maturation attained by the parasites within the red cells and that persistence of the parasites, if verified, means that schizogony takes place in some more fixed and static kind of cell than the lymphocyte.

In this connection it is interesting to note that Nuttall (1913) proved that *B. canis* persists 3 years in the dog, and *Nuttallia equi* for the same period in the horse; he states that *B. bigemina* also persists in the blood of cattle for years after recovery. How this parasite manages to remain has never been explained. It may be a case of repeated invasion of a few new corpuscles, for its length of life in all of them is strictly limited by the time the mature red cells themselves exist in the body. In man a conservative maximum estimate would place the duration of life of the corpuscles at 70 days; in cattle it cannot be much longer. Perhaps a developmental phase of this parasite

remains to be discovered in some other kind of cell. In the case of *B. canis* those endothelial and other cells which ingest trypan blue would come under suspicion first.

We agree with Wenyon that the classification of Theileriidae and Babesiidae is at present in a very confused state and includes several genera which are probably not valid. The reason is that there are too few criteria for distinction. Observations concerning size, shape and number are of value because of their objective nature and the ease with which they can be made; but the host has some influence on the parasite. In some cases of East Coast fever *T. parva* is distinctly larger than in others. Reliance on the method of division of intra-erythrocytic stages, though safe in the case of most Babesiidae, is not justified in the case of any Theileriidae because the actual method of division has not been ascertained through the study of living specimens (Nuttall, Fantham and Porter, 1910). The cells in which schizogony occurs require to be identified beyond question. What the parasites do must also be taken into consideration. Fortunately there has been gratifying advance in this direction. In all probability the relationships of the Theileriidae and the Babesiidae will remain obscure until their life cycles in the arthropod vectors have been determined. Except for the experimental observation as to the inheritance of some species through the eggs the life cycles are only known of two forms, *T. parva* and *B. bigemina*.

VI. Summary.

The history of the ticks (*Rhipicephalus appendiculatus*) employed for transmission of East Coast fever is detailed in Part I. During the *1st day* the ticks generally become attached and are partly held in place by a kind of cement substance (Fig. 1).

Penetration usually occurs at some time during the *2nd day*, and the cellular elements of the corium quickly respond. There is a local swelling of the capillary endothelium, an infiltration with polymorphonuclear leucocytes and a slight increase in eosinophiles. More cement substance is produced which hardens about the edges. Some of it enters through the wound and spreads out in the corium along lines of least resistance (Fig. 2).

Later on the eosinophiles increase greatly in number and in turn give place to macrophages which ingest some of the cement substance. Mast cells also become more numerous. The area of reaction extends 2 or 3 mm. from the tip of the proboscis. Marked cytolysis of fibroblasts, of histiocytes and of the swollen endothelial cells occurs. The latter leads to haemorrhage (Figs. 6, 8, 9). The red blood cells and leucocytes are, however, not destroyed. They are sucked up by the tick, the leucocytes first because they accumulate before the haemorrhages take place.

Twenty-five of the sixty-six ticks examined were found to contain parasites in their salivary glands, but no parasites were seen in the sixty-six pieces of skin excised while the ticks were attached. Soon after the parasites enter the

blood stream and spread throughout the system the corium of the skin is invaded by them like other parts of the body (Table III).

The medium-sized lymphocytes are the principal cells parasitised; but parasites are also found in lymphoblasts, large and small lymphocytes and occasionally in parenchymatous liver cells. We never saw them in vascular endothelial cells. For this reason we are unable to substantiate the view, which has apparently never been seriously questioned, that they undergo schizogony in this situation. The first agamonts were noted in lymphocytes in gland smears the day the fever first exceeded 103° F. The gamonts appeared about a day later and the intra-erythrocytic forms soon after. Taking the average of a number of cases: (1) The temperature first surpassed 103° F. on the 9·8th day after infestation with ticks. (2) The first Koch bodies within lymphocytes were observed on the 14·8th day, that is to say, 5 days after the fever commenced. (3) The first parasites within the red cells were noted 1·6 days later, on the 16·4th day after infestation.

The cycle which the parasite undergoes in changing from the agamonts to the intra-erythrocytic forms is illustrated in Diagram II (fully described on p. 36).

The alterations in the tissues caused directly or indirectly by the parasitic invasion were studied principally in specimens secured at autopsy from animals killed *in extremis*. The changes which apparently dominate relate to the lymphatic system. The medium-sized lymphocytes and to a less extent the small and large lymphocytes and lymphoblasts, first in the lymph glands and later in all parts of the body, become infested with Koch bodies which grow in their interior apparently without causing any severe injury. The distribution of Koch bodies is recorded in Table III, and their incidence in the lymph glands and in the liver in Tables IV and V. The evidence presented does not support the current conception that the parasites serve as a powerful stimulus to the multiplication of lymphocytes. The enlargement of lymph glands is caused more by oedema than by increase in their number. But there is a marked redistribution of lymphocytes. Their number is reduced in the lymph glands and in the peripheral blood stream and increased in the blood vessels of the liver and some other organs. Perivascular lymphocytic infiltrations are also a notable feature of the reaction in the liver, kidneys, adrenals and several other organs, but they are of rare occurrence in the nervous system. It is uncertain how far they are formed by the emigration of lymphocytes from the blood stream and by the proliferation of lymphocytes previously present perivascularly.

Small haemorrhages constitute a second alteration of importance. They are particularly prone to occur in the epicardium and endocardium, but may be found in almost any part of the body. Evidence is lacking that these haemorrhages play a part in the genesis of the perivascular lymphocytic infiltrations. The latter are far more widespread and are chiefly observed in locations where haemorrhages are seldom seen.

Oedema is a third type of reaction. It is constantly found in the lymph glands and occasionally in the lungs, especially in the later stages of the disease.

Generally speaking, therefore, the lesions reported in this paper are acute in type. The functional activity of the several organs (except the lymph glands) has not been impaired sufficiently severely or over a time long enough to leave distinct structural changes in the parenchymatous organs; but the specimens examined were not collected from protracted cases of East Coast fever or from animals under suspicion of having undergone repeated attacks of the disease.

It is the parasites in the red blood cells which are capable of continuing the cycle if they are ingested by ticks able to act as vectors. We have recognised three principal groups: (1) small spherical-ovoid parasites which are the first to make their appearance, (2) tailed parasites, and (3) plump forms, both of which develop later and become more numerous than the spherical-ovoid parasites. Possible stages of transition are represented in Diagram I (p. 37). Though many parasites undergo differentiation and some probably divide, a variable number degenerate within the erythrocytes.

We question the validity of the genera into which the family Theileriidae are customarily divided on account of the inadequacy of the data on which they are based.

REFERENCES.

Barron, E. S. G. and Harrop, G. A. Jr. (1929). Studies on blood cell metabolism. *J. Biol. Chem.* **84**, 89.

Bettencourt, A., França, C. and Borges, J. (1907). Un cas de Piroplasmose bacilliforme chez le Daim. *Arch. Inst. Roy. de Bact. Camara Pestana*, **1**, 341.

Bevan, E. W. (1924). East Coast fever—the theory of latency. *Trans. Roy. Soc. Trop. Med.* **18**, 328.

Bruce, Sir David (1910). *Amakebe.* A disease of calves in Uganda. *Proc. Roy. Soc.* ser. B, **82**, 35.

Brumpt, E. (1924). Les piroplasmes des bovidés. I. Les Theileries. *Ann. Parasit. Hum. et Comp.* **2**, 340.

Bulger, H. A., Dixon, H. H., Barr, D. P. and Schregardus, D. (1930). The functional pathology of hyperparathyroidism. *J. Clin. Inv.* **9**, 143.

Carpano, M. (1915). Piroplasmosi tipo "parvum" nei bovine del basso bacino del Mediterraneo. *La Clin. Vet.* **38**, 497 and 553.

Carrel, Alexis and Ebeling, H. H. (1922). Pure cultures of large mononuclear leucocytes. *J. Exper. Med.* **36**, 365.

Christophers, S. R. (1906). The anatomy and histology of ticks. *Sci. Mem. Med. and Sanit. Depts. Gov. of India*, N.S. No. **23**, 55 pp.

Cleland, J. B. (1910). A note on Eosinophile cells in the exudate from tick-bites on a horse. *Proc. Roy. Soc. Med.* **3**, part 3, 42.

Collaud, L. (1906). Beiträge zur pathologischen Histologie der Niere bei Rhodesian Redwater der Rinder in Südafrika. *Inaugural Diss.*, Zürich, 36 pp.

Cooper, W. F. and Laws, H. E. (1915). Some observations on the practice and theory of dipping. *Parasitology*, **8**, 190.

COWDRY, E. V. (1926). Geographic distribution of spontaneous encephalitis in rabbits. *J. Exper. Med.* **43**, 725.

—— (1928). The microchemistry of nuclear inclusions in virus diseases. *Science*, **68**, 40.

COWDRY, E. V. and HAM, A. W. (1930). The life cycle of the parasite of East Coast fever in ticks transmitting the disease (Preliminary note). *Ibid.* **72**, 461.

—— —— (1932). Studies on East Coast fever. I. The life cycle of the parasite in ticks. *Parasitology*, **24**, 1.

DENNIS, E. W. (1932). The life cycle of *Babesia bigemina* (Smith and Kilbourne) of Texas cattle fever in the tick *Margaropus annulatus* (Say). *Univ. of Calif. Publ. in Zoology*, **38**, No. 11, 263.

DU TOIT, P. J. (1918). Zur Systematik der Piroplasmen. *Arch. f. Protistenk.* **39**, 84.

—— (1931). Immunity in East Coast fever. *Rep. Dir. of Vet. Serv. and Animal Husb.* No. **17**, part 1, 3.

FLEXNER, S. and AMOSS, H. L. (1917). The relation of the meninges and choroid plexus to poliomyelitic infection. *J. Exper. Med.* **25**, 525.

FRANÇA, C. (1913). Quelques considérations sur le genre Theileria et description d'une nouvelle espèce de ce genre (*Theileria stordii*). *Centralbl. f. Bakt.* Orig. **67**, 171.

GONDER, R. (1911). The development of *Theileria parva*, the cause of East Coast fever of cattle in South Africa. *Rep. Gov. Vet. Bact.* Pretoria, p. 69.

—— (1911 *a*). Die Entwicklung von *Theileria parva*, dem Erreger des Küstenfiebers der Rinder in Afrika. Teil II. *Arch. f. Protistenk.* **22**, 170.

GOODPASTURE, E. W., WOODRUFF, A. M. and BUDDINGH, G. J. (1931). The cultivation of vaccine and other viruses in the chorio-allantoic membrane of chick embryos. *Science*, **74**, 371.

GRAY, C. E. and ROBERTSON, W. (1903). Redwater in Rhodesia. *Agric. J. of Cape of Good Hope*, **21**, 435.

HARRIS, W. H. (1932). Histopathological effects of Thorium dioxide on lymphatic glands of animals visualised by the Menville-Ané method. *Proc. Soc. Exp. Biol. and Med.* **29**, 1049.

HEGNER, R. W. (1923). The effects of changes in diet on the incidence, distribution and number of certain intestinal protozoa of rats. *Amer. J. Hyg.* **3**, 180.

—— (1926). Biology of host-parasite relationships among protozoa. *Quart. Rev. Biol.* **1**, 393.

HEGNER, R. W. and MACDOUGALL, M. S. (1926). Modifying the course of infections with bird malaria by changing the sugar content of the blood. *Amer. J. Hyg.* **6**, 602.

HOEPPLI, R. and FENG, L. C. (1931). Histological reactions in the skin due to ecto-parasites. *Nat. Med. J. of China*, **17**, 541.

KLEINE, F. K. (1908). Bemerkung zu Dr Mayer's Arbeit. *Arch. f. Schiffs- u. Tropen-Hyg.* **11**, 494.

KNUTH, P. and DU TOIT, P. J. (1921). Tropenkrankheiten der Haustiere. *Handb. d. Tropenkr.* Leipzig: J. A. Barth, 2nd ed. **6**, 361.

KOCH, R. (1897). Über die Viehseuchen in Deutsch-Ostafrika. *Deutsch. Kolon.-Bl.* **24**, 719.

—— (1903). Report on Rhodesian red-water or African coast fever. *J. Comp. Path. and Therap.* **16**, 273.

DE KOCK, G. and QUINLAN, J. (1926). Splenectomy in domesticated animals and its sequelae, with special reference to anaplasmosis in sheep. *Rep. Dir. Vet. Ed. and Res.* Pretoria, Nos. **11** and **12**, part 1, p. 369.

LICHTENHELD, G. (1911). Beurteilung eines Befundes von Koch'schen Plasmakugeln in Niereninfarkten einer Elenantilope. *Z. f. Infektionskr. d. Hausth.* **9**, 154.

LUCAS, M. S. (1930). Results obtained from applying the Feulgen reaction to Protozoa. *Proc. Soc. Exp. Biol. and Med.* **27**, 258.

MAXIMOW, A. (1928). Cultures of blood leucocytes. *Arch. f. exper. Zellforsch.* **5**, 169.

MAXIMOW, A. (1930). *Textbook of Histology*, 833 pp. (edited by W. Bloom). Philadelphia: W. R. Saunders Co.

MAXIMOW, A. and BLOOM, W. (1932). The lymphocytes and plasma cells (revised by W. Bloom). *Special Cytology*, 2nd ed., **2**, 603. New York: Paul B. Hoeber.

MAYER, MARTIN (1910). Über das ostafrikanische Küstenfieber der Rinder. *Arch. f. Schiffs- u. Tropen-Hyg.* **14**, 1.

METTAM, R. W. M. (1924). Snotziekte in cattle. *Rep. Dir. Vet. Ed. and Res.* Pretoria, Nos. **9** and **10**, p. 395.

MEYER, K. F. (1911). Notes on the nature of Koch's granules and their rôle in the pathogenesis of East Coast fever. *Rep. Gov. Vet. Bact.* Pretoria, Union of South Africa, 1910, p. 56.

MONTGOMERY, R. E. (1913). On bodies resembling Koch's bodies in the spleen of a topi (*Damaliscus corrigum jimela*). *Ann. Rep. Vet. Path. Lab.* Nairobi, 1911–12, 25.

MOSKWIN, J. A. (1929). Die Wirkung des Bisses der Zecke *Ornithodorus papillipes* Bir. auf die Haut der Versuchstiere. *Centralbl. f. Bakt. etc.*, Abt. 1, Orig. **110**, 208.

MOTTRAM, J. C., CRAMER, W. and DREW, A. H. (1922). Vitamins, exposure to radium and intestinal fat absorption. *Brit. J. Exp. Path.* **3**, 179.

NUTTALL, G. H. F. (1913). The Herter Lectures. III. Piroplasmosis. *Parasitology*, **6**, 302.

NUTTALL, G. H. F. and FANTHAM, H. B. (1910). *Theileria parva*, the parasite of East Coast fever in cattle. *Ibid.* **3**, 117.

NUTTALL, G. H. F., FANTHAM, H. B. and PORTER, A. (1910). Observations on *Theileria parva*, the parasite of East Coast fever. *Ibid.* **2**, 325.

NUTTALL, G. H. F. and HINDLE, E. (1913). Conditions influencing the transmission of East Coast fever. *Ibid.* **6**, 321.

NUTTALL, G. H. F. and STRICKLAND, C. (1908). On the presence of an anticoagulin in the salivary glands and intestines of *Argas persicus*. *Ibid.* **1**, 302.

PAWLOWSKY, E. N. and STEIN, A. K. (1927). Experimentelle Untersuchungen über die Wirkung von *Ixodes ricinus* (Ixodidae) auf die Menschenhaut. *Arch. f. Schiffs- und Tropen-Hyg.* **31**, 574.

PERLA, D. and MARMORSTON-GOTTESMAN, J. (1930). Further studies on *Trypanosoma lewisi* infection in albino rats. *J. Exp. Med.* **52**, 601.

PFEIFFER, L. (1891). *Die Protozoen als Krankheitserreger*, 2nd ed. Jena.

ROBINSON, L. E. and DAVIDSON, J. (1913). The anatomy of *Argas persicus*. Part II. *Parasitology*, **6**, 217.

ROSS, P. H. (1911). *Nairobi Laboratory Reports for* 1904–10. East Africa Protectorate, I.

SABBATANI, L. (1898). Fermento anticoagulante dell' *Ixodes ricinus*. *Arch. Ital. de Biol.* **31**, 37.

SAWYER, W. A. and LLOYD, W. (1931). The use of mice in tests of immunity against yellow fever. *J. Exper. Med.* **54**, 533.

SCHILLING, CL. (1930). Immunität bei Protozoeninfektionen. In Kolle and Wassermann's *Handb. d. path. Mikroorg.* **8**, 95.

SCHILLING, CL. and MEYER, K. F. (1930). Piroplasmosen. *Ibid.* **8**, part 1, 1.

SCHWETZ, J. and STORCK, N. (1930). *Theileria* (*Gonderia*) *mutans* et corps bleus de Koch. *Bull. Soc. d. Path. exot.* **23**, 377.

SERGENT, EDM. (1920). Étude morphologique du *Piroplasma* (*Gonderia*) *mutans* du Bœuf. *Ann. Inst. Pasteur*, **35**, 193.

SERGENT, EDM., DONATIEN, A., PARROT, L. and LESTOGUARD, F. (1929). Sur l'existence de corps en grenade dans le cycle évolutif de *Gonderia mutans*. *Bull. Soc. Path. Exot.* **22**, 542.

SERGENT, EDM., DONATIEN, A., PARROT, L., PLANTUREUX, E. and ROUGEBIEF, H. (1927). La "fièvre de la côte orientale" et la Theileriose nord-africaine. *Ibid.* **41**, 489.

STECK, W. (1928). Histological Studies on East Coast fever. *Rep. Dir. Vet. Ed. and Res.* Pretoria, Nos. **13** and **14**, 243.

STRICKLAND, C. (1915). Observations on the blood in East Coast fever of cattle. *Parasitology*, **8**, 244.

TALIAFERRO, W. H. (1928). Infection and immunity in bird malaria. *Porto Rico Rev. Pub. Health and Trop. Med.* **4**, 155.

TEICHMANN, E. and BRAUN, H. (1911). Ueber ein Protozoentoxin (Sarkosporidiotoxin). *Arch. Protistenk.* **22**, 351.

THEILER, SIR ARNOLD (1905). How long an area remains infected with East Coast fever. *Transvaal Agric. J.* **4**, 700.

—— (1908). The influence of cold on ticks and Piroplasma parvum. *Bull. Soc. path. exot.* **1**, 451.

—— (1911). The artificial transmission of East Coast fever. *Rep. Gov. Vet. Bact.* Pretoria, p. 7.

THEILER, SIR ARNOLD, GRAY, C. E. and POWER, W. H. (1915). Diseases transmitted by ticks. *10th Internat. Vet. Congress*, London, **3**, 806.

THEILER, SIR ARNOLD and GRAF, H. (1928). *Gonderia mutans* or *Theileria mutans*. *13th and 14th reports Dir. Vet. Ed. and Res.* Pretoria.

THEILER, MAX (1930). Susceptibility of White Mice to the Virus of Yellow Fever. *Science*, **71**, 367.

WALKER, J. (1930). *Aids to Stockowners. Dept. of Agriculture*, Kenya Colony, 158 pp.

WALKER, J. and WHITWORTH, S. H. (1929). *Artificial immunisation and immunity in their relation to the control of East Coast fever.* Nairobi, Kenya: Government Printer, 18 pp.

WENYON, C. M. (1926). *Protozoology*, **2**, 779–1563. London: Baillière, Tindall and Cox.

ZWICK, W., SEIFRIED, O. and WITTE, J. (1929). Weitere Beiträge zur Erforschung der Bornaschen Krankheit des Pferdes. *Arch. wiss. u. prakt. Tierheilk.* **59**, 511.

EXPLANATION OF PLATES I–X.

The photographs were all taken by W. B. C. D. of Giemsa stained preparations, but at various magnifications: Figs. 1–8, ×300; Figs. 9–12, ×1700; and Figs. 13–24, ×300. The following reference letters are used in Figs. 1, 3 and 5

D = finely dentate sheath of chelicera.
H = hypostome.
S = shaft of chelicera.
X = exudate with indentations corresponding to denticles on sheath of chelicera.

PLATE I.

Fig. 1. Site of tick (*Rhipicephalus appendiculatus*) bite on *2nd day* after attachment to ears of calf No. 5838. Shows mouth parts of tick but no penetration into corium.

Fig. 2. Section taken a little to one side of the penetrating mouth parts of a tick the *3rd day* after attachment to calf No. 5869. The cement substance has greatly increased in amount and has made its way down into the corium.

PLATE II.

Fig. 3. Communication between the tick and the subepithelial tissue on the *4th day* after attachment to calf No. 5914. The communicating space in the middle contains many leucocytes.

Fig. 4. Shows the kind of reaction usually seen in the deeper parts of the corium at this time (*4th day*). The endothelium is somewhat swollen and the capillaries are loaded with polymorphonuclear leucocytes and eosinophiles. Both types of cells also occur extravascularly, the former predominating as in the blood vessels.

PLATE III.

Fig. 5. Hypostome and chelicerae (in trans. sect.) surrounded with cement substance in the corium cut parallel with the surface of the epithelium on the *5th day* after attachment to calf No. 5869. Note that the lamination of the cement substance is as noticeable in this situation as on the surface of the epidermis.

Fig. 6. Intensified cellular reaction in the corium near the point of penetration (illustrated in the upper right portion of the photomicrograph) likewise on the 5th day of attachment to the same calf. The leucocytes are here more abundant outside of the capillaries, many of which have broken down.

PLATE IV.

Fig. 7. The *7th day* after attachment of a tick to calf No. 5840. Section to the side of the penetrating parts. There has been cytolysis of the histiocytes of the corium with destruction of capillaries and haemorrhage. The local accumulation of leucocytes is, however, less marked than on the 5th day (Fig. 6).

Fig. 8. From another site of bite on the same calf on the same day. The cytolysis and the local haemorrhage is greater.

PLATE V.

Fig. 9. Lymph gland smear from calf No. 5869 made on the *8th day* after infestation with ticks. In the centre of the photomicrograph is a medium-sized lymphocyte which contains a single parasite in the pale-staining area of cytoplasm beneath its nucleus. This is the earliest form which we were able to discover in infected calves.

Fig. 10. A dividing lymphocyte in a gland smear taken from the same calf on the *14th day* after infestation. To the left of the chromosomes is illustrated an agamogenous Koch body possessed of five nuclei. These nuclei are of unequal size. The most elongated one below and to the right was apparently at the time of fixation nearing the point of segmentation into two of the size of the one adjoining it on the left.

Fig. 11. Gland smear from bovine No. 5774 (not included in our regular series) made in an advanced stage of the disease. The lymphocyte above contains a Koch body in which large, agamogenous and small, gamogenous stages of the parasite can be recognised; while in the lymphocyte just below it the Koch body (to the right of the nucleus) is made up entirely of the agamogenous forms. In the lowermost lymphocyte no parasites are visible.

Fig. 12. This is from the same smear as Fig. 11. Instead of a "combination" Koch body it shows, in about the centre, two Koch bodies which are respectively wholly agamogenous and gamogenous in nature. The first is above and to the right of the nucleus of lymphocyte which contains the latter. It is a large, roughly spherical, deeply staining body in which many irregular nuclei are to be seen. The second encircles the nucleus of the lymphocyte to the right below and to the left. The nuclei in it are smaller, more regular in shape and stain more intensely.

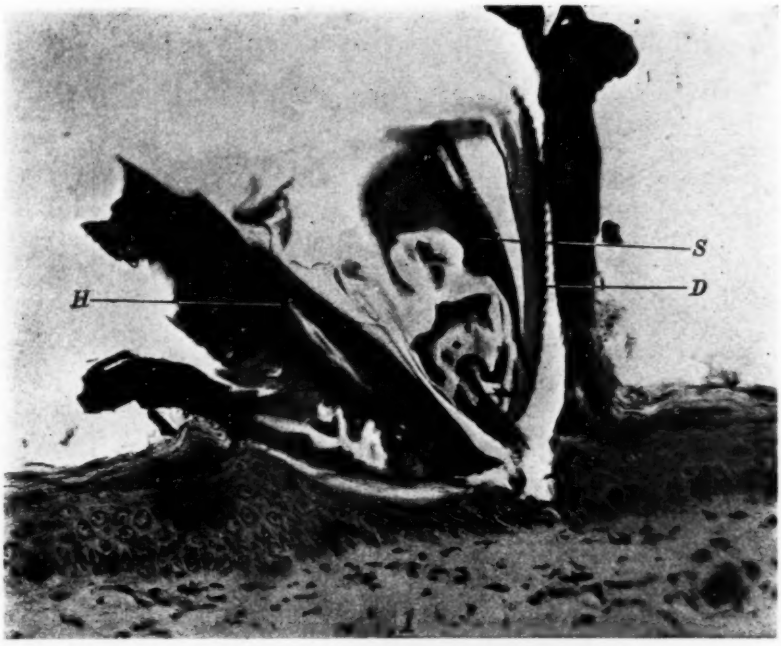
PLATE VI.

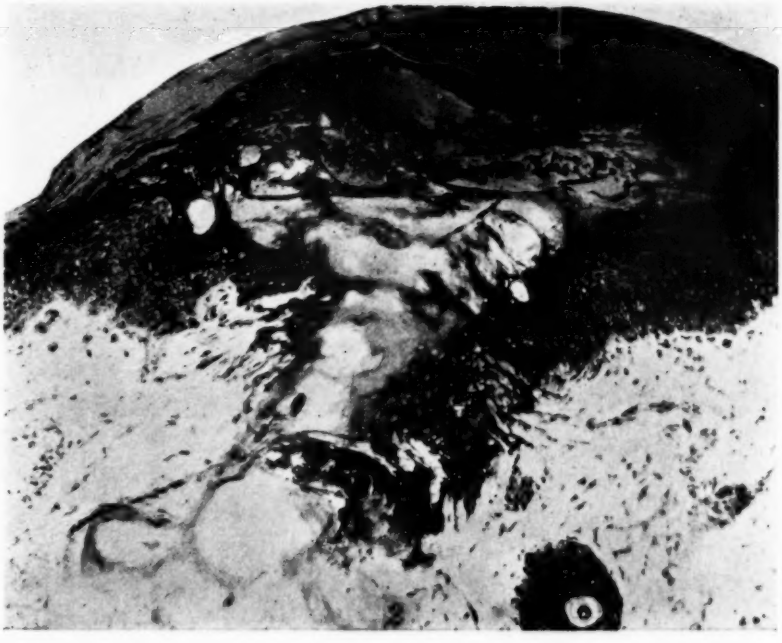
Fig. 13. Section of the cortical portion of an anterior mediastinal lymph gland from calf No. 5838, taken the *16th day* after infestation with infected ticks. Shows part of follicle with germinal centre a little below and to the left. Above and to the right can be seen a portion of the peripheral lymph sinus. The chief visible deviations from normal are the increase in extent of the centre and the decrease in small lymphocytes about it.

Fig. 14. Section of an anterior mediastinal lymph gland from the same calf. The area illustrated is more in the medullary part of the gland away from the germinal centres. It also shows a decrease in the number of lymphocytes.

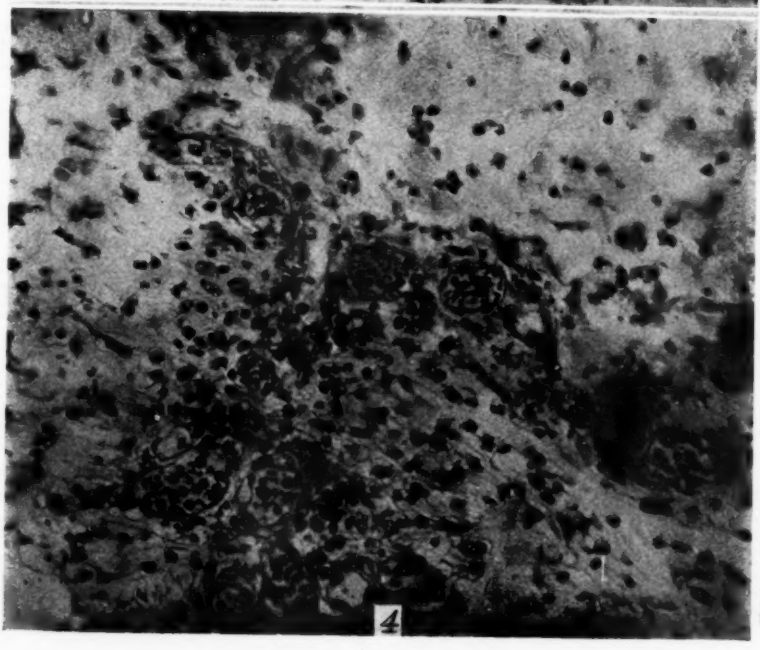
Fig. 15. This photomicrograph also illustrates the depletion of the lymphoid tissue. A medullary cord pursues a winding course upward in approximately the middle of the figure. From the same lymph gland as Fig. 13.

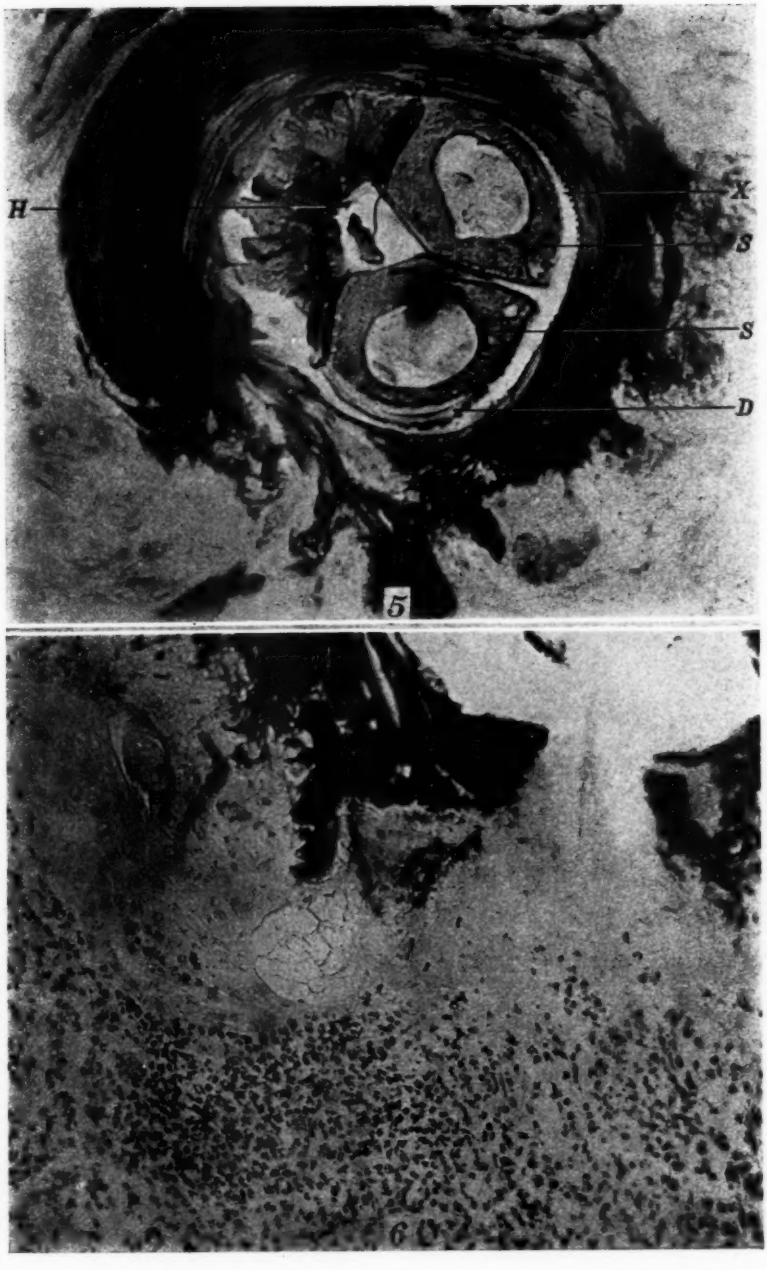
Fig. 16. Shows a small island of small and medium-sized lymphocytes still persisting in a portion of the medulla almost denuded of them. From the same lymph gland as Fig. 14.

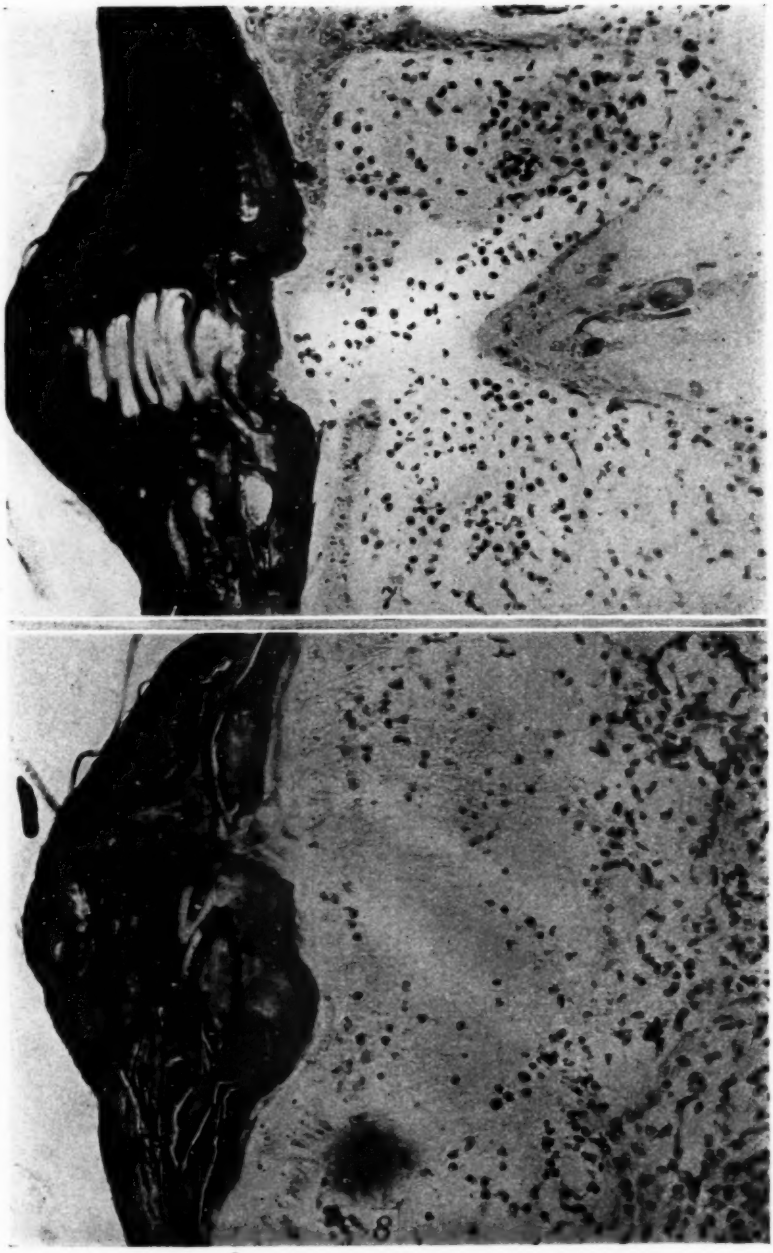

S
D
H

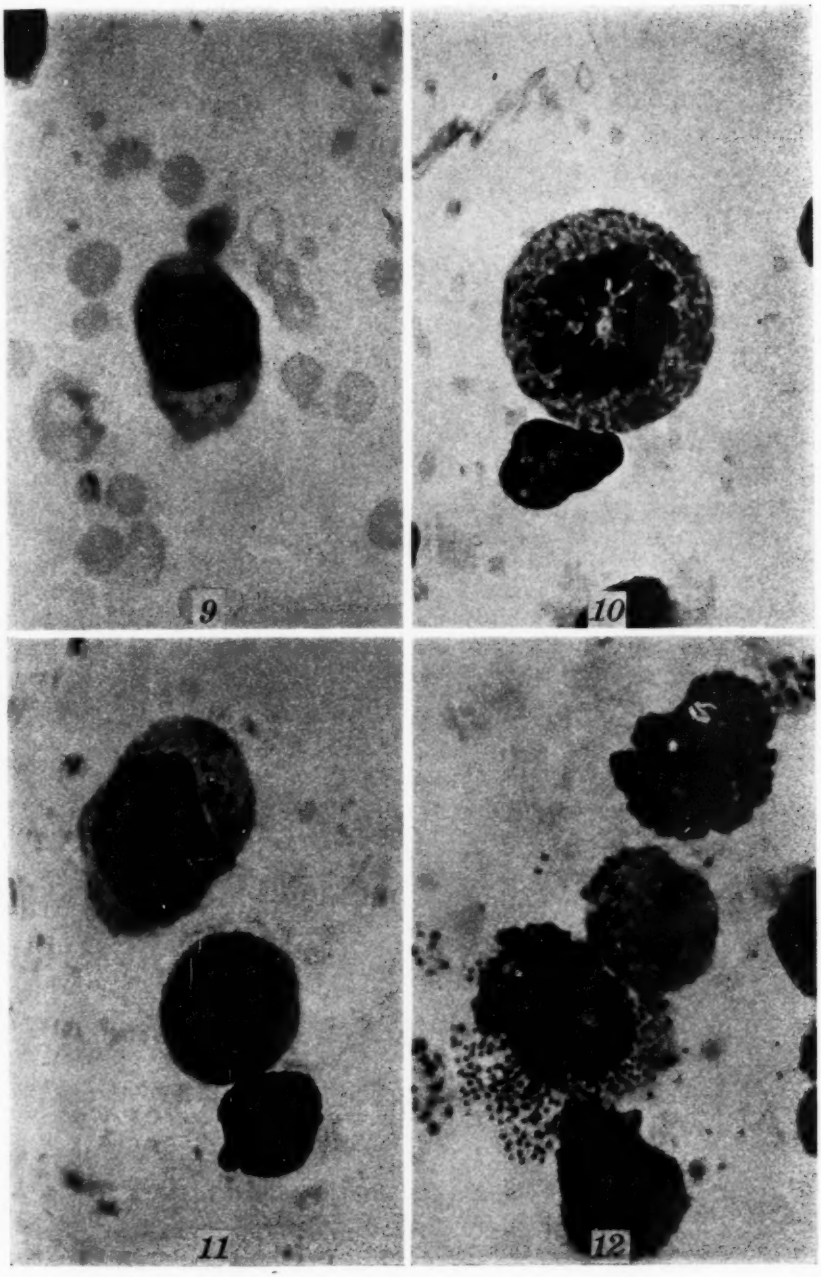


X
D
3


4


H
X
S
S
D
5
6


7
8


9
10
11
12

PARA

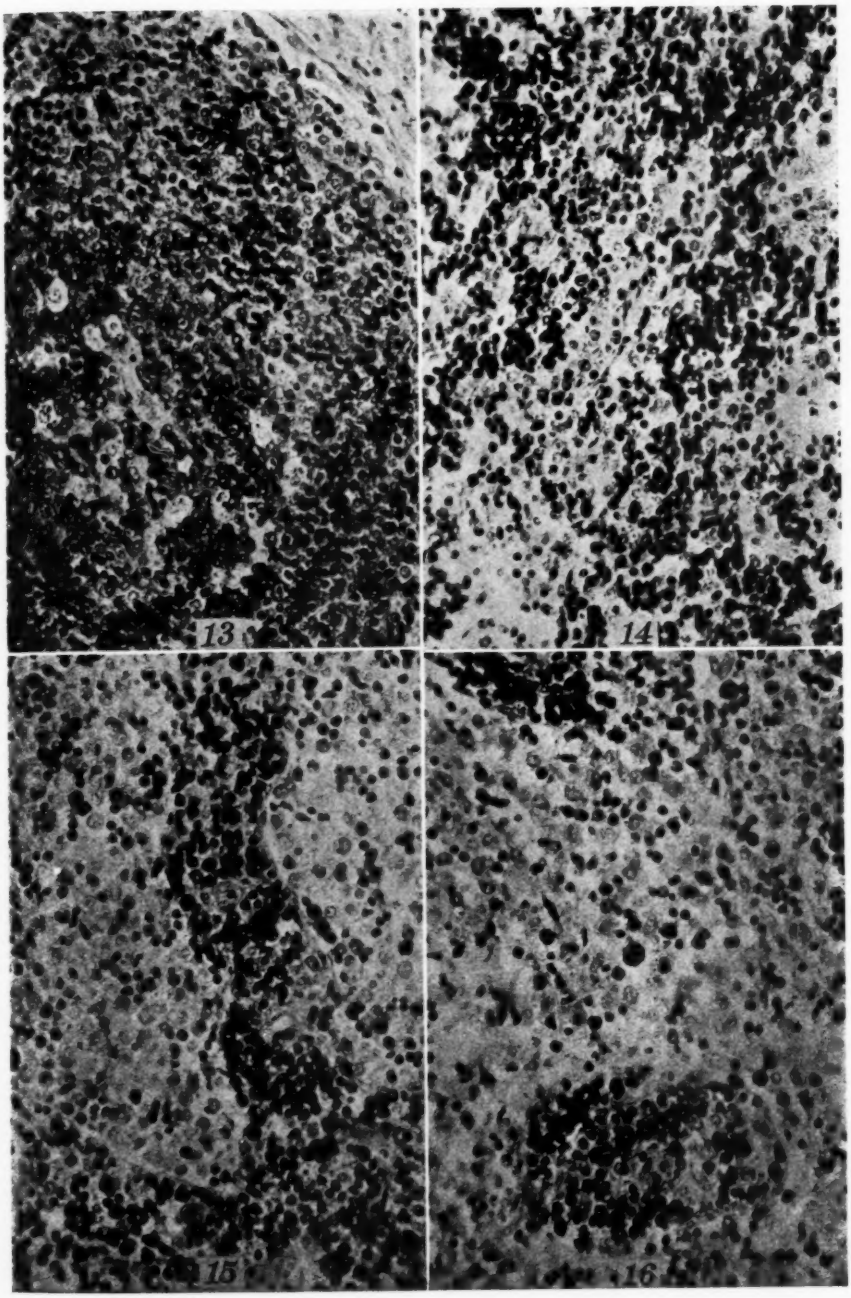

13
14
15
16

PLATE VII.

Fig. 17. Section of lymph gland from bovine No. 7600, taken 22 *days* after exposure to infected ticks and eleven days after first rise in temperature. The animal was killed *in extremis*. This figure representing the maximum change, should be compared with Fig. 13 of an early stage. Both are of comparable parts, namely a germinal centre with neighbouring peripheral lymph sinus. It will be noted (1) that the tissue between the germinal centre on the right and the sinus on the left is reduced in extent and contains few if any lymphocytes, (2) that the peripheral lymph sinus has become greatly dilated.

Fig. 18. Section from lymph gland of bovine No. 7644, also 22 *days* after exposure to infected ticks and eleven days after first rise in temperature. Killed *in extremis*. It is from the medullary portion and shows (1) an increase in connective tissue elements, (2) an increase in blood vessels and (3) a swelling of their endothelial lining.

Fig. 19. Portion of another section of a lymph gland of the same bovine. In about the centre is illustrated a germinal centre, in which, like that represented in Fig. 17, the usual large, pale-staining cells active in the production of lymphocytes are replaced by deeply staining ones, many of which are picnotic and degenerating.

Fig. 20. Taken from a section of the medullary part of a lymph gland of the same bovine. It shows the development of strands of connective tissue and the almost complete washing out from the gland of lymphocytes. Interstitial fibrosis is often much more marked than is illustrated in this figure.

PLATE VIII.

Fig. 21. Small area of section of liver of bovine No. 7600. Slightly below the centre a large lymphocyte is represented within one of the hepatic sinusoids. In the lower left part of the cytoplasm of this lymphocyte a gamogenous Koch body is visible. Since its various nuclei are on different planes they have not all been photographed with equal distinctness. Some appear darker than others, but their size and shape are similar to those of the nuclei of the gamogenous Koch body photographed from a smear (Fig. 12). Above and to the right of the lymphocyte is a part of another Koch body of the same kind.

Fig. 22. Small vessel seen in the wall of the gall bladder of the same bovine. Below and to the left is a cell containing a large number of gamogenous forms of the parasite.

Fig. 23. Portion of section of kidney of bovine No. 7644 illustrating the character of the infiltrating cells between the tubules on the right and on the left. Three Koch bodies are distinctly shown. The one on the upper left contains relatively large and irregularly shaped nuclei provided with cytoplasm which colours intensely and is accordingly classified as an agamogenous form. The gamogenous one in the lower left exhibits tiny well-defined nuclei. The Koch body to the right exhibits both irregular, large nuclei and small, deeply staining ones, and may be described as a mixed form. The distinction between the two types of nuclei is, however, better seen in Fig. 11, which represents their appearance in a smear.

Fig. 24. Part of section of heart muscle of bovine No. 7600 illustrating Koch bodies between the fibres. Of the two slightly above the centre, the one to the left is agamogenous, while the one to the right is gamogenous. The Koch body in the extreme lower left corner of the photomicrograph is gamogenous.

PLATE IX.

These figures were all drawn with the aid of Zeiss apochromatic objective 1·3 mm., compensating oculars No. 20 and camera lucida at a magnification of 3250 diameters at the level of the table from sections cut at 4 μ and stained by Giemsa's method.

Fig. 25. A small lymphocyte containing an agamogenous Koch body from the right inguinal lymph gland of calf No. 5840.

Fig. 26. A larger lymphocyte from the same lymph gland in which the individual nuclei of the agamogenous Koch body are much larger. Some of them appear to have been fixed in a state of division.

Figs. 27 and 28. Two lymphoblasts from a germinal centre of the same gland. Note the smaller relative amount and the typical arrangement of their nuclear chromatin. Both contain rather feebly developed agamogenous Koch bodies.

Fig. 29. A much hypertrophied medium-sized lymphocyte from the lymph gland of bovine No. 7537. The contained Koch body is also of the agamogenous variety. It apparently occupies the entire cytoplasmic area.

Fig. 30. A typical gamogenous Koch body within a medium-sized lymphocyte from a lymph gland belonging to the same calf. The individual nuclei are much smaller and are provided with traces of cytoplasm. The cytoplasm of the lymphocyte, which was originally basophilic in nature, has almost disappeared; only a roughly spherical blue staining mass can be distinguished above and slightly to the right of the nucleus. This is the remains of the centrosomal complex.

Fig. 31. A cell of the same kind from bovine No. 6657 which contains a large gamogenous Koch body and the vestiges of a blue staining centrosomal complex. The nucleus of this lymphocyte, however, was undergoing degeneration by karyorrhexis.

Fig. 32. Another cell from the same gland of the same bovine equipped with a gamogenous Koch body. This cell may be also a lymphocyte, but in a more advanced stage of degeneration, or a polymorphonuclear leucocyte.

Fig. 33. A medium-sized lymphocyte from a lymph gland of calf No. 5840. It contains several agamogenous Koch bodies. Its cytoplasm has lost its natural basophilic properties.

Fig. 34. A large lymphocyte in the kidney of the same calf. This lymphocyte occupies the lumen of a capillary at its point of branching. It contains a single agamogenous Koch body, the growth of which has not noticeably injured its cytoplasm.

Fig. 35. A medium-sized lymphocyte from a perivascular infiltration in the kidney of the same calf. The nucleus (to the left) is very picnotic. The individual nuclei of the agamogenous Koch body were evidently preserved in a condition of rapid multiplication.

Fig. 36. A medium-sized lymphocyte resembling the one illustrated in Fig. 33, but from a perivascular infiltration in the wall of the colon of calf No. 5840.

Fig. 37. A disintegrating lymphocyte from a perivascular infiltration in the liver of bovine No. 6657. Its nucleus is picnotic and its cytoplasm is disappearing, the Koch body being fragmented and liberated.

Fig. 38. A large binucleated lymphocyte from a similar location in the same bovine, containing a small gamogenous Koch body in the usual position.

Fig. 39. A large lymphocyte with kidney-shaped nucleus and two small gamogenous Koch bodies from a perivascular infiltration in the liver of calf No. 5840.

Fig. 40. A portion of a parenchymatous liver cell of bovine No. 6657, the upper and lower parts of which are not illustrated on account of lack of space. Above its nucleus is seen a small lymphocyte possessed of a tiny gamogenous Koch body. The lymphocyte is surrounded by a clear space indicating that it has been received by the liver cell as a sort of foreign body.

Fig. 41. A part of another parenchymatous liver cell of the same animal, the sides of which are omitted. It contains clumps of gamonts presumably set free by the destruction of a Koch body.

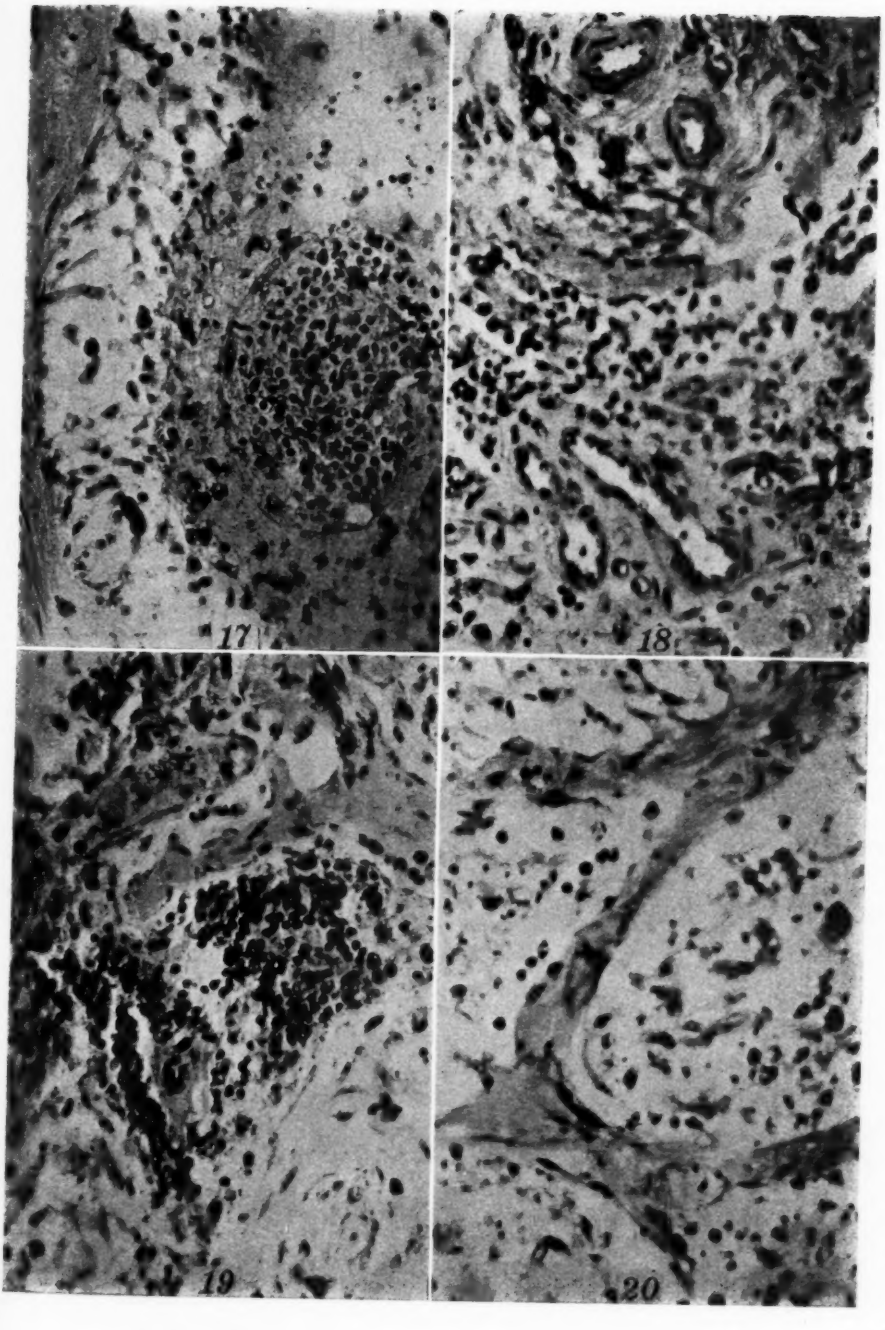
PLATE X.

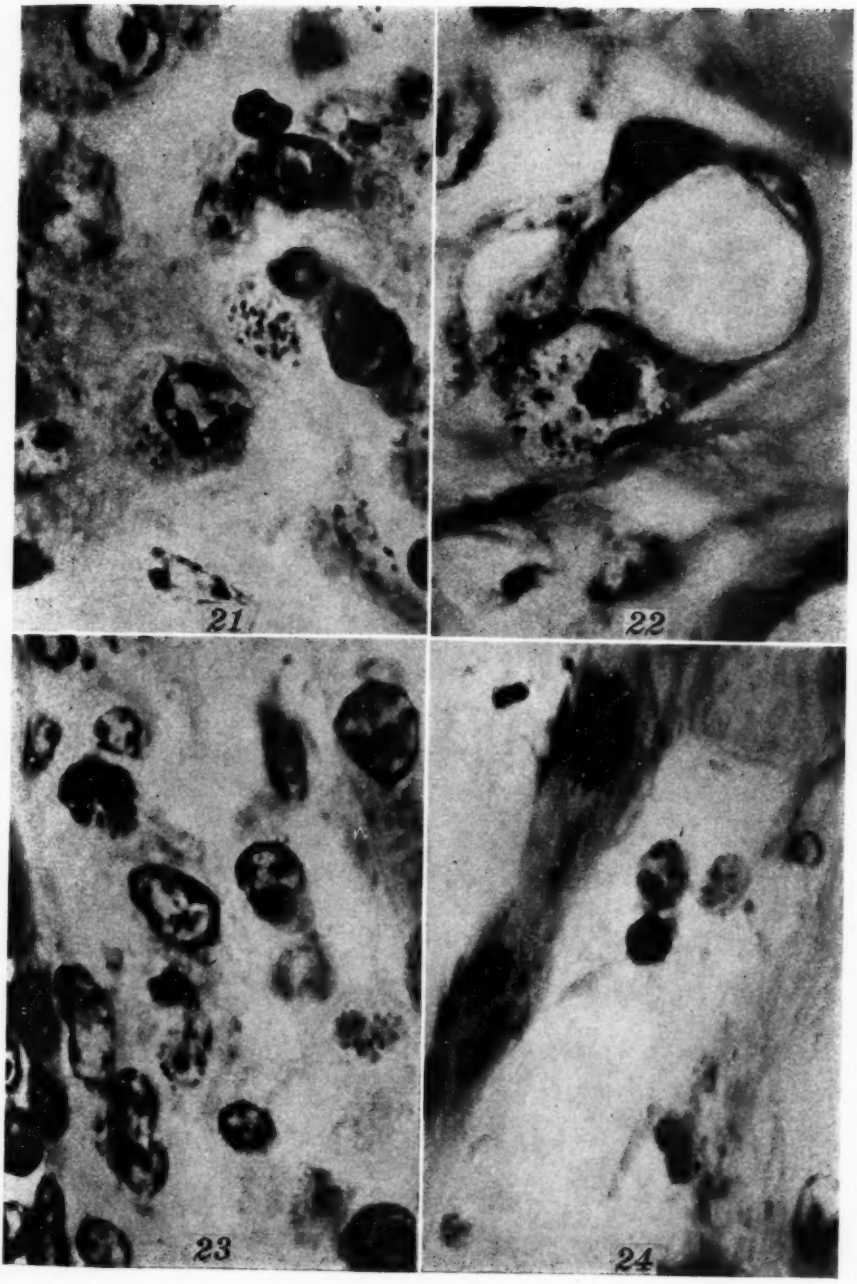
All these figures represent drawings made in the same way of red blood cells as seen in smears of the peripheral blood. Figs. 42–67 are from calf No. 5840 (see Tables I to VIII).

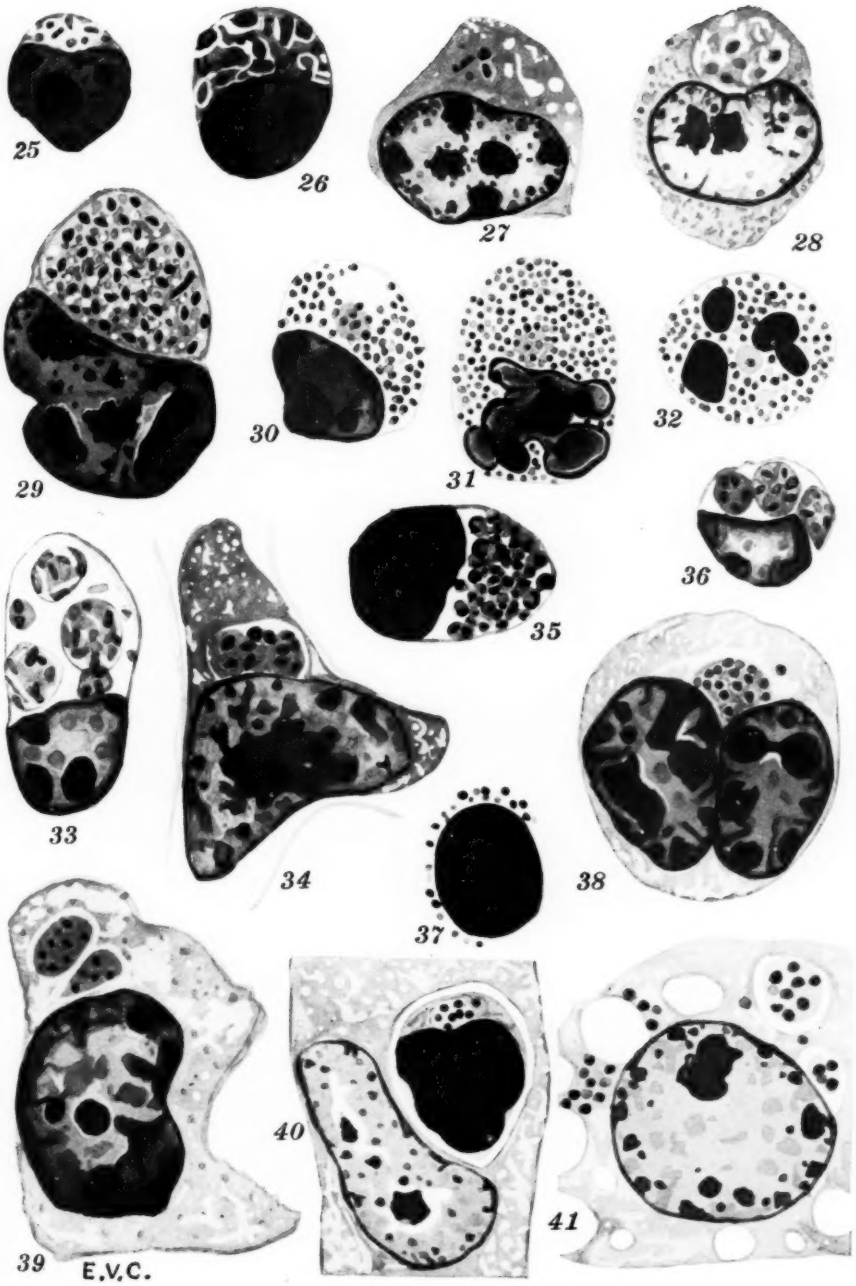
Fig. 42. Shows the only form of the parasite observed on the 16*th day* after infestation. It resembles a gamont.

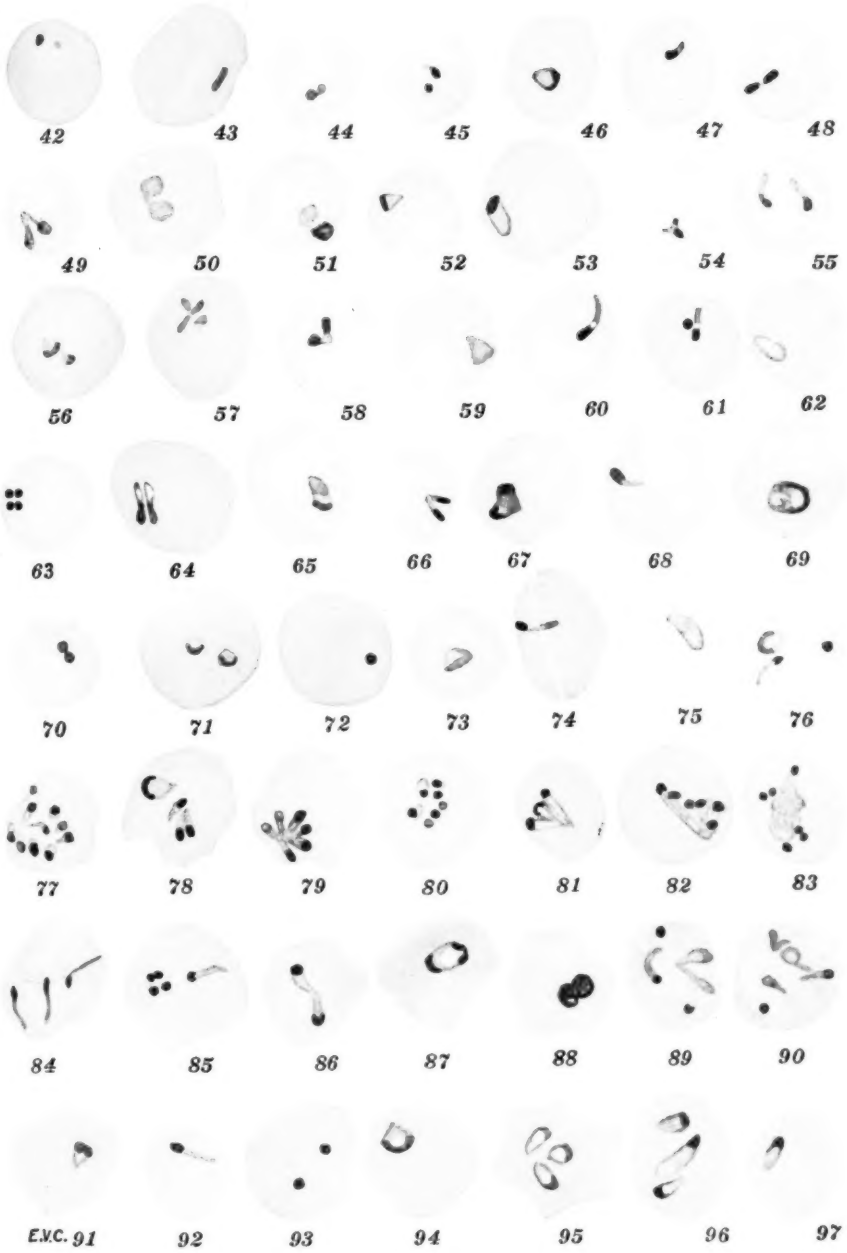
Figs. 43 and 44 are from a smear made the 17*th day*. In the first, the parasite is noticeably longer, while in the second a stage suggestive of division is presented.

Figs. 45–47 illustrate three red cells on the 18*th day*. In Figs. 45 and 47 forms possessed of a tail-like appendage of blue staining cytoplasm are shown. Fig. 46 illustrates the first of the plump, more rounded parasites to be seen in this calf.


17
18
19
20


21
22
23
24


25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
E.V.C.
40
41


42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
E.N.C. 91
92
93
94
95
96
97

Figs. 48–55 are of cells taken the 19*th day*. They show the appearance at this time of a variety of forms. The V type (Fig. 49) is rare, also the triangle type (Fig. 54). Forms with longer tails are more numerous (Fig. 55), likewise the plump types (Figs. 50–53). Some of the latter exhibit signs of degeneration with loss of characteristic chromatin staining.

Figs. 56–62 are from the 20*th day*. They include the first cross form observed (Fig. 57), longer tailed forms (Fig. 60), V types (Fig. 58) and plump forms (Figs. 56, 59 and 62), some of which are obviously degenerative.

Figs. 63–69 are from the 21*st day*. They comprise parallel pairs (Fig. 63), V types (Fig. 66), plump forms (Figs. 65, 67 and 69), tailed parasites single (Fig. 68) and together degenerating (Fig. 64).

Figs. 70–76 are from smears made on the 22*nd day* when the animal was killed for examination though it was not *in extremis*. The forms already illustrated are shown and one cell (Fig. 76) containing three parasites, the largest number observed in this calf.

Figs. 77–83 are from calf No. 7539 and represent a very heavy infection.

Figs. 84–90 are from calf No. 5740 (see Tables VI and VIII).

Figs. 91–97 are from bovine No. 6673 (see Tables III, VI and VIII).

(*MS. received for publication* 19. VIII. 32.—Ed.)

SOME OBSERVATIONS ON THE VALUE AND THE MECHANISM OF THE RED-CELL ADHESION TEST FOR TRYPANOSOMIASIS OF MAN AND OTHER ANIMALS.

By A. WORMALL[1], D.Sc.

From the Institute for Human Trypanosomiasis Research, Entebbe, Uganda.

THE red-cell adhesion phenomenon, described first by Duke and Wallace (1930), involves the adhesion of red blood corpuscles to trypanosomes under the influence of a substance (adhesin) which appears in the blood of an animal suffering from trypanosomiasis. This reaction is similar in most respects to the "thrombocytobarin" or "Rieckenberg reaction," the adhesion phenomenon with platelets observed by Rieckenberg (1917) and studied later by Brussin and Beletzky (1925), Kritschewsky and Tscherikower (1925), Davis and Brown (1927), Leupold (1928), and more recently by Regendanz and Jurukoff (1930). Johnson and Lester (1928–9), following the suggestion of Davis and Brown that this reaction might "be of service in the diagnosis of trypanosomiasis and in the discovery of game reservoirs in Africa and other countries," made an extensive study of platelet adhesion in Nigeria. They tested the plasma of 200 trypanosomiasis patients, and reached the conclusion that this test has a very definite value as an aid to the diagnosis of human trypanosomiasis and that it is much more reliable than the formol-gel test for this infection. With a small percentage of their definitely established cases of human trypanosomiasis, however, negative results were obtained with this adhesion test, and, in addition, several others gave doubtful reactions. Saunders (1931) carried out platelet adhesion tests on cases of human trypanosomiasis in the Gold Coast, and concluded that when virulent strains of trypanosomes are used, 91 per cent. of proved cases of trypanosomiasis show some degree of adhesion, while 75 per cent. of cases not proved to be trypanosomiasis give completely negative reactions. This author concludes that false positives with this test may be due to non-specific reactions or sub-infective inoculations or to mild or relapsing infections.

After comparing red-cell and platelet adhesion, Duke and Wallace reached the conclusion that "red cell adhesion is a manifestation of the adhesion phenomenon already described by other workers." There are, however, certain aspects in which the two phenomena appear to differ; thus red-cell adhesion, which occurs only with the red cells of primates or with the red cells of a small percentage of guinea-pigs which have been infected with trypanosomes,

[1] Seconded from the Department of Physiology, the University of Leeds.

appears to be more specific in this respect than the platelet test in which many different indicators can be used (cf. Davis and Brown, 1927). Another difference noted by Duke and Wallace related to the properties of a certain monkey plasma which gave very powerful red-cell adhesion, but failed to give any adhesion with platelets, bacteria or yeasts. Until further and more exhaustive comparisons are made it would appear impossible, however, to decide how far these two phenomena are related. Leupold (1928) noticed in her experiments on platelet adhesion that single red cells often adhered to the trypanosomes, and considered that the platelets adhere in greater numbers because of their smaller weight. This occasional adhesion of single red cells in tests with rodents' blood does not appear to be the same, however, as red-cell adhesion with the cells of primates, in which tests the whole trypanosome is usually covered with red cells. As pointed out by Duke and Wallace (1930) the choice of blood platelets or red corpuscles does not depend upon the relative specific gravities, since plenty of platelets are always present in the red-cell adhesion tests, but they rarely adhere to the trypanosomes.

A further study of red-cell adhesion by Wallace and Wormall (1931) revealed the fact that, in addition to the adhesin, some other factor is required for the reaction; this other factor is present in the serum and plasma of most if not all normal animals, and shows such a strong resemblance to haemolytic complement that it might be permissible to conclude that the two substances are identical. No evidence has yet been obtained which militates against the view that this "X factor" is complement, but since it is impossible to establish as yet the identity of the two substances, the term "X factor" or complement-like substance will be used throughout this paper.

Red-cell adhesion may thus be observed when the following are present in the test solution: (*a*) the red blood corpuscles of any primate, (*b*) an adhesin (present in the serum or plasma of an animal which is, or recently has been, infected with trypanosomes), (*c*) trypanosomes, preferably related to those which gave rise to the production of the adhesin, and (*d*) a complement-like component of the serum or plasma of any normal animal.

This test has a high practical value, since in the first place it can be carried out very easily and in almost every case there is no possible doubt when a positive reaction is obtained. Control reactions with "normal" individuals carried out by Duke and Wallace (1930) gave readings up to 5 or even 6 per cent., whereas the majority of trypanosomiasis patients gave 40–100 per cent. adhesion. The conclusion was reached that a positive reaction invariably indicates recent or actual infection with trypanosomes, but, on the other hand, a single or even a number of negative observations do not exclude trypanosomiasis. As a result of the introduction of certain modifications (Wallace and Wormall, 1931), the non-specific adhesion can be reduced still further, and in the investigations described in this paper the majority of sera and plasmas from control men and monkeys gave no adhesion or, at the most, 1 or 2 per cent. adhesion. Furthermore, if tests are always carried out

with several strains of trypanosomes, as is advocated later in this paper, the chances of wholly negative reactions with the serum or plasma of an infected animal are reduced considerably. The number of cases of human trypanosomiasis studied more completely, *i.e.* tested at frequent intervals with several strains of trypanosomes, is not very high, but it is significant to note that in no instance in this series of tests did the plasma of a sleeping sickness patient or that of a monkey suffering from trypanosomiasis fail to give good adhesion with at least one strain of trypanosomes.

This red-cell adhesion test has proved extremely useful in connection with other laboratory studies and clinical investigations on trypanosomiasis at Entebbe, and a considerable amount of data has been collected. All the evidence thus obtained indicates that it has a very definite value as a laboratory test in addition to its value under certain circumstances as a field test for human trypanosomiasis. In its original form, however, the red-cell adhesion test had, like the platelet adhesion test, marked limitations. The not infrequent occurrence of "non-specific" adhesion with the plasma of individuals who do not suffer from trypanosomiasis, and the failure, on occasions, to obtain adhesion with established cases of this disease, detract considerably from the value of a test of this nature. Further investigations have been carried out, therefore, in an attempt to increase the value of this test for diagnostic purposes. The investigations described here were concerned mainly with attempts (*a*) to reduce, and if possible abolish, "non-specific" adhesion, (*b*) to increase the percentage of positive results with established cases of trypanosomiasis, and (*c*) to determine whether it would be possible to introduce such modifications as would allow the test to be made with sera which had been collected several days previously. In addition, investigations on the mechanism of this phenomenon of red-cell adhesion have been continued, and are, in part, described here.

Experimental details.

The red-cell and trypanosome suspensions and the diluted plasma were prepared by the methods described by Wallace and Wormall (1931). In most of the tests described in this paper, the source of adhesin was the diluted plasma, or the serum, of men and monkeys suffering from trypanosomiasis, the diluted plasma being obtained by mixing one volume of blood with two volumes of citrate-Ringer-glucose solution and centrifuging to remove the red cells. On a few other occasions, sheep adhesin sera were used.

Unless otherwise stated, the tests made involved the mixing in a small test-tube of equal volumes of the following:

(*a*) A suspension of washed human red cells (of blood group O)—the red cells from six or seven drops of human blood in about 1 c.c. of citrate-Ringer-glucose solution.

(*b*) Diluted adhesin plasma or undiluted serum (of infected man or monkey).

(*c*) Trypanosome-containing plasma ("tryp. plasma")—the diluted plasma of an infected animal (guinea-pig or monkey).

As in the previous work the results are recorded as a percentage of adhering trypanosomes to total trypanosomes counted.

The cases of human trypanosomiasis were diagnosed by the detection of trypanosomes in the gland juice or in the blood: all but two of the patients examined in the series of tests described here showed, at some time or other, trypanosomes in both the gland juice and in the blood. The two exceptions failed to show trypanosomes in the blood. The previous history and the subsequent treatment of most of these patients are recorded elsewhere (Wormall, in press 1932).

Examination of trypanosomiasis patients at the laboratory (Entebbe) and some observations on the best method of making the tests.

Repeated examinations have been made with the serum or plasma of fourteen patients kept for varying lengths of time in the laboratory hospital at Entebbe, and on a large number of "normal" individuals. Tests were carried out on each patient immediately on arrival at Entebbe and at fairly regular intervals during his stay there. Some of these patients had had previous treatment with tryparsamide or Bayer 205, whereas others had received no drug treatment; all were given intravenous injections of Bayer 205 during their stay at Entebbe, this treatment usually starting 2 or 3 weeks after arrival at the laboratory. On every occasion the serum or plasma from each patient gave at least one strongly positive result when adhesion tests were carried out with several strains of trypanosomes. The results of these investigations are, in general, similar to those of Duke and Wallace (1930), who applied the test to over 200 cases of trypanosomiasis in Uganda, and it is not considered necessary to give a full list of the tests. A few typical experiments are quoted, however, to illustrate the main conclusions. Tests were also made later with twelve sera which had been obtained from trypanosomiasis patients in the West Nile area of Uganda: these sera were preserved by the addition of phenol and were brought back to Entebbe for subsequent examination. The results of a few of the tests with these preserved sera are given in Table V and are discussed later.

Tables I, II and III include a few tests which show the marked variations which may occur from time to time in the reaction between the plasma of any trypanosomiasis patient and a given strain of trypanosome. Trypanosomes undoubtedly show considerable variations in power of adhesion during their life in an experimental animal, and on occasions they gave no adhesion when tested with an adhesin serum with which they previously or subsequently gave very good adhesion (cf. also Duke and Wallace, 1930). On the other hand, there have been occasions on which the trypanosomes in a certain animal have given good adhesion with an adhesin serum (*a*) and little or none

with adhesin serum (*b*), whereas tests at a subsequent date gave a converse result. A complete reversal of this type is, however, not very common.

In the work described in this paper these variations in the power of different strains of trypanosomes to give adhesion were particularly noticeable in a series of similar tests with the sera of infected monkeys, a few of these results being given in Table IV, and in the tests made with the sera collected from

Table I.

Diluted plasma of patient	*T. gambiense* (strain G)			*T. gambiense* (strain D)
	%	%	%	%
Sumba	54	31	98	67
Orachi	84	22	—	41
Otenyi	92	1	—	14
Date	2. i. 31	7. i. 31	2. ii. 31	7. i. 31

Table II.

Diluted plasma of patient		*T. gambiense* (various strains)					*T. rhodesiense*	
		D_1 %	D_2 %	H %	F %	G %	Strain X %	Strain D %
17. iv. 31	Lisuwa	21	47	4	0	69	4	—
	Kibuka	15	3	0	1	9	47	—
	Normal	0	0	0	0	0	0	—
21. iv. 31	Lisuwa	16	—	—	16	44	11	0
	Kibuka	13	—	—	14	5	50	11
	Normal	0	—	—	0	0	0	0
21. iv. 31	Monkey serum 835 (*R*)	0	—	—	0	0	40	3

Note. D_1 and D_2 refer to different guinea-pigs infected with the same strain of *T. gambiense*. Serum 835 was the serum of a monkey which had been infected with *T. rhodesiense*.

Table III.

Serum of patient	*T. gambiense*				*T. rhodesiense*
	Strain D %	Strain D %	Strain G %	Strain Y %	Strain X %
Achan	95	74	47	32	0
Atoyo	35	21	10	70	0
Umu	39	60	81	33	0
Normal	0	0	0	0	0

Note to Tables I, II and III. These tables give the results, as percentage adhesion, of some of the adhesion tests with various plasmas (and sera) of sleeping sickness patients and different strains of trypanosomes.

Tests. Red-cell suspension + human serum or plasma + "tryp. plasma" (see p. 67 (*c*)).

trypanosomiasis patients in the West Nile area of Uganda (cf. Table V). The results with the stock monkey adhesin sera support the view that there is a considerable degree of specificity, since the *rhodesiense* adhesin sera reacted only slightly, and in some instances not at all, with strains of *T. gambiense* and *vice versa*. Although the specificity is by no means absolute, it is usually possible to determine whether trypanosomes are of the *gambiense* or of the

rhodesiense type by the use of suitable adhesin sera. In relation to this question of the specificity of the reaction, the results with the plasma and serum of the patient Kibuka present certain interesting features. This patient was the only one of those investigated in this series who gave good adhesion with *T. rhodesiense*, and although some adhesion occurred with *gambiense* strains, the plasma of Kibuka always gave much better adhesion with a certain strain of *T. rhodesiense*; indeed this plasma usually gave much better adhesion with this last-named strain than did the adhesin sera from monkeys which had been infected with the same strain. In view of the recent discovery by Duke (1930) of a case of *T. rhodesiense* infection in man in Uganda, it was decided to make a further study of Kibuka in order to determine whether or not this was another instance of infection with *T. rhodesiense*. Unfortunately, however, the patient decided to return to his home before an extensive study could be made, and all attempts to isolate the trypanosome were unsuccessful. Trypanosomes had been detected in the gland juice and also in the blood of this patient on one occasion prior to his arrival at Entebbe, but none could be detected during the six weeks that he was under our investigation. Attempts were made by Dr A. R. D. Adams to isolate the trypanosome by injecting some of the blood of this patient into six mice, but none of these animals developed any trypanosome infection.

As a result of these studies on human cases of trypanosomiasis the conclusion is reached that it is necessary to use for each test several strains of *T. gambiense* and *T. rhodesiense*. The testing of each serum or plasma with at least three, and preferably four or five, different strains of *T. gambiense* is advisable, and whenever there appears to be the possibility of a *T. rhodesiense* infection a similar number of *rhodesiense* strains should be used. When one, or preferably two, good positive reactions (greater than 20 or 25 per cent.) are obtained with any serum, it is not necessary, of course, to carry out further tests on this serum with other strains of trypanosomes. Since trypanosomes, especially certain strains, show marked variations in their power to give adhesion, it is useful to have available stock adhesin sera (human or monkey) corresponding to the various strains used for the tests. In this way, it is quite easy to determine whether the trypanosomes in a certain animal are suitable or not for adhesion tests. Under these conditions, a much larger percentage of positive reactions with the serum or plasma of trypanosomiasis patients is obtained, and it is believed that there will be few instances in which entirely negative results occur with the serum of an individual who undoubtedly suffers from trypanosomiasis.

A further improvement may be introduced in doubtful cases by administering trypanocidal drugs to the patient. Usually it is not necessary to administer these drugs before carrying out adhesion tests, and excellent adhesion can almost invariably be obtained with the serum of untreated trypanosomiasis patients. With two of the patients investigated in this work, however, the intravenous injection of 1 g. of Bayer 205 resulted in a very marked improve-

ment in the percentage adhesion; adhesion to the extent of about 20 per cent. only was obtained before treatment, whereas tests carried out a week after the administration of Bayer 205 gave 50–90 per cent. adhesion. In the other cases, where excellent adhesion was obtained before drug treatment was started, the injection of Bayer 205 similarly led to an improvement in the extent of adhesion, but here the difference was, of course, less pronounced. This increase in adhesin content as a result of the injection of Bayer 205 has been noted repeatedly in the case of monkeys, but here again this treatment does not appear to be at all necessary and some of the most powerful adhesin sera have been obtained from monkeys which have received no drug treatment. Johnson and Lester (1928–9), in their study of the platelet adhesion test, also reached the conclusion that a short course of treatment with a trypanolytic drug converts a doubtful or weak reaction into one which is more intense, if the case is one of trypanosomiasis.

Thus tests of this nature have considerable advantages over certain other diagnostic tests, since it is not necessary to withhold treatment until trypanosomes are detected in the blood or in the gland juice. Suspected cases can be treated with trypanocidal drugs and samples of serum collected a week or so later and used for adhesion tests.

Examination of sera preserved with phenol and kept at ordinary temperatures.

Whenever adhesion tests are made with a number of sera from trypanosomiasis patients and one strain of trypanosome, a relatively large number of the tests may be slightly positive only or may even be negative, and the necessity for using several strains of trypanosomes renders the test more difficult for field purposes. Experiments have been made therefore to determine how far the test can be applied to sera which have been preserved by the addition of an antiseptic. No difficulty is experienced in obtaining good adhesion with a human trypanosomiasis serum which has been collected with the usual aseptic precautions and which has been allowed to stand for 1 or 2 days at the ordinary temperatures in Entebbe (23–26° C.) and a moderate amount of contamination does not interfere with the reaction. If the test is to be of more general use, however, much longer intervals may elapse between the collection of the serum and the carrying out of the tests and some antiseptic will be required to prevent gross contamination. In the experiments recorded below, phenol was the preservative used, and although this investigation may be regarded as a preliminary study only, the results are sufficiently encouraging to warrant further examination.

Investigations in the laboratory at Entebbe had shown that phenol may be regarded as suitable for this purpose, and an opportunity occurred during a trip to the West Nile district of the Uganda Protectorate to collect serum from a few sleeping sickness patients. Two or three c.c. of blood were withdrawn from a vein by syringe and placed in a sterile test-tube. On the following day the serum was withdrawn, phenol added to the extent of 1 volume of 2·5 per

cent. phenol solution to 10 volumes of serum, and the mixture placed in a small ampoule which was then sealed up. These sera were kept at the ordinary temperature (23–26° C.) and were tested 17–19 days after the date of collection of the blood. Satisfactory adhesion was obtained with each serum (cf. Table V), whilst the sera of normal individuals collected at the same time and treated in the same way gave entirely negative results.

Table IV.

Monkey adhesin serum	Adhesion with strains of *T. gambiense*			Adhesion with strains of *T. rhodesiense*	
	Strain D	Strain F	Strain L	Strain W	Strain Y
	%	%	%	%	%
731 (*G*)	84	98	11	0	0
751 (*G*)	9	86	27	0	5
640 (*G*)	91	95	81	0	10
809 (*R*)	0	0	9	96	100
835 (*R*)	0	0	4	49	—
Normal	0	0	0	0	0

Tests. Red-cell suspension + adhesin serum + "tryp. plasma." *G* = *T. gambiense* infection. *R* = *T. rhodesiense* infection.

Table V. *Adhesion with human adhesin sera preserved with phenol.*

	T. gambiense			*T. rhodesiense*
	Strain Z	Strain Y	Strain L	Strain X
	%	%	%	%
Serum (1)	0	55	70	1
,, (2)	1	90	97	1
,, (3)	0	99	97	0
,, (4)	1	47	80	3
Normal	0	0	0	0

Tests. Red-cell suspension + human serum + "tryp. plasma." Sera 1, 2, 3 and 4 were from trypanosomiasis patients. These sera were collected in the West Nile district (Uganda) on June 27th, 1931, and the tests in the table above were made on July 16th, 1931.

The addition of this amount of phenol to the adhesin serum does not appear to have any deleterious effect on the trypanosomes used in the test, and trypanosomes in the mixtures were usually quite active 2 or even 3 hours after the test had been set up. When double this quantity of phenol was used, the results were not so satisfactory and the sera gave much less adhesion: the suggestion is made that this is possibly due to a destruction or inactivation of the antibody-like adhesin by the higher concentration of phenol, for the immobilisation of the trypanosomes in these adhesion tests does not appear to be very considerable.

Adhesion with the red cells of infected guinea-pigs.

In the early studies on red-cell adhesion, adhesion was obtained with the red cells of infected guinea-pigs (Duke and Wallace, 1930, p. 448), but later experiments have shown that this phenomenon is rather rare (Wallace and Wormall, 1931). On certain occasions, however, adhesion of this type does occur, and an experiment of this nature is recorded in Table VI.

Table VI.

Washed cells	Adhesin serum	Trypanosomes	Adhesion %
Human	Sheep 15 (absorbed)*	Guinea-pig plasma (H)	100
Sheep	" "	" "	3
Guinea-pig (normal)	" "	" "	2
Guinea-pig (infected—H)	" "	" "	65
Guinea-pig (infected—Y)	" "	" "	2
Human	Sheep 10 (absorbed)*	Guinea-pig plasma (Y)	94
Sheep	" "	" "	0
Guinea-pig (normal)	" "	" "	0
Guinea-pig (infected—H)	" "	" "	8
Guinea-pig (infected—Y)	" "	" "	0

* These sera were absorbed for 1 hour at room temperature (about 25° C.) with a mixture of washed normal human and guinea-pig red cells in order to remove interfering agglutinins.

Adhesion was obtained with human red cells and with the red cells of the infected guinea-pig (H) from which the trypanosomes used for the tests were taken, whereas the red cells of normal guinea-pigs and those of guinea-pigs infected with other strains of trypanosomes did not adhere to any significant extent. Another sheep adhesin serum (sheep 10—strain Y) gave only very slight adhesion (8 per cent.) when tested with the red cells of the infected guinea-pig (H) and trypanosomes of strain Y, and no significant adhesion with the same trypanosomes and the red cells of another infected guinea-pig (strain Y). Tests made on another day with the same animals and adhesin sera gave similar results, and there seems to be a certain degree of consistency in the phenomenon: the red cells of some infected guinea-pigs thus appear to have acquired the property of adhering to the homologous trypanosomes when a corresponding adhesin serum is added.

Titration of adhesin sera and the amount of complement (or "X factor") required for the reaction.

Experiments were carried out to determine to what extent human and monkey adhesin sera can be diluted without disappearance of the power to induce adhesion. A typical example is given in Table VII (series A) together with a similar series of tests (series B) in which a fixed amount of guinea-pig plasma was added to each tube. From these results it will be seen that if no extra complement is added, moderate dilution of the adhesin serum results in marked loss of the power to give adhesion: when extra complement is added, however, the adhesin serum can be diluted to a much greater extent before loss of the power to give adhesion occurs. Satisfactory adhesion can usually be obtained, therefore, when the concentration of the adhesin serum in the final mixture is no more than 1 : 64, provided extra complement is added. In this respect, the red-cell reaction appears, therefore, to be similar to those immunological reactions which are brought about by an immune body plus complement, for there appears to be a close interdependence between complement on the one hand and the immune body, or adhesin, on the other. Excess of complement can, to some extent, compensate for a deficiency in

immune body, and with less complement more immune body (or adhesin) is required: the adhesin thus appears to behave like a typical immune body.

Table VII. *Titration of adhesin serum with and without extra complement.*

	Concentration of adhesin serum (in final mixture)						
Series	1 : 4 %	1 : 8 %	1 : 16 %	1 : 32 %	1 : 64 %	1 : 128 %	1 : 256 %
A	54	23	10	3	0	0	0
B	95	82	56	21	17	14	7

Series A. Cells (human) + adhesin serum + dilute trypanosome plasma + citrate-Ringer-glucose.

Series B. Cells (human) + adhesin serum + dilute trypanosome plasma + dilute guinea-pig plasma (1 : 3).

Table VIII. *Amount of "X factor" necessary.*

Cells	Adhesin	Trypanosomes	"X factor" (guinea-pig plasma)	Adhesion %
Human	Monkey serum 761	Washed trypanosomes (M. 787)	None	0
,,	,, ,,	,, ,,	Plasma diluted 1 : 3	98
,,	,, ,,	,, ,,	,, ,, 1 : 6	93
,,	,, ,,	,, ,,	,, ,, 1 : 12	74
,,	,, ,,	,, ,,	,, ,, 1 : 24	4
,,	,, ,,	,, ,,	,, ,, 1 : 48	0

Other titrations have been carried out to determine the amount of complement or "X factor" required for adhesion, and for this purpose it was necessary to use adhesin sera and trypanosomes which were as far as possible free from complement. The adhesin sera (human and monkey) were therefore filtered through Berkefeld filters, and the absence of any significant amount of complement demonstrated by haemolytic tests, whilst the trypanosomes were washed well with citrate-Ringer-glucose solution to remove all traces of guinea-pig plasma. The results (cf. Table VIII) showed that the complement-like "X factor" takes a very active part in this reaction and that a small quantity only is required. Thus guinea-pig plasma diluted to 1 : 48 in the final solution will still induce powerful adhesion in an appropriate trypanosome-adhesin-red-cell system; beyond this point the titration curves usually show a sudden fall.

Several authors have produced evidence that complement is required for the platelet adhesion phenomenon, and Brown and Davis (1927) observed that the presence of complement is essential for the adhesion of bacteria to leptospira, and that minute amounts of complement are sufficient for this purpose.

Some properties of the adhesin.

The adhesin has been studied from the point of view of resemblance to other immune bodies, and although the results do not invariably appear to be consistent with this view, there is usually a very close relationship between the two groups. In some of these studies, the influence of dialysis on the adhesin

content of adhesin sera was investigated. Human and monkey adhesin sera were placed in parchment dialysing thimbles and these thimbles immersed in a large volume of 0·9 per cent. NaCl solution and kept in the ice chest for 24 hours, the external fluid being changed twice during this period. These dialysed sera were then tested in various dilutions for adhesin power and compared with corresponding strengths of undialysed serum. The results, recorded in Table IX, indicate that the adhesins, like true antibodies, are relatively non-dialysable.

Table IX. *Adhesion with dialysed adhesin serum.*

	Concentration of adhesin serum (in final mixture)					
Adhesin	1 : 4 %	1 : 8 %	1 : 16 %	1 : 32 %	1 : 64 %	1 : 128 %
Serum 640	98	90	79	69	48	18
Dialysed serum 640	95	91	84	70	44	19
Serum 731	98	—	65	—	48	8
Dialysed serum 731	98	—	70	—	46	9

Tests. Adhesin serum + human red-cell suspension + "tryp. plasma" + dilute guinea-pig plasma.

Discussion.

These investigations have served to demonstrate the high value of the red-cell adhesion test for the diagnosis of trypanosomiasis in man and certain other animals. Under satisfactory conditions, a large number of tests can readily be carried out in the laboratory with each serum (or diluted plasma) and several strains of trypanosomes, and usually there is not the slightest difficulty in deciding whether the test is positive or negative. A single negative, or even a number of negative results, does not, according to Duke and Wallace (1930), exclude trypanosomiasis, but, as a result of a later series of tests on men and monkeys, the present author has reached the conclusion that if the tests are made, not with one strain of trypanosome only, but with at least three different strains of trypanosomes of the same species, and if suitable tests are made simultaneously to demonstrate that these trypanosomes are capable of giving adhesion at that time, there will be very few instances where negative results are obtained with undoubted cases of trypanosomiasis in man or monkey. If this can be fully established, the test will have a greatly enhanced value, and more reliance could be placed on a negative or preferably a series of negative results.

Red-cell adhesion tests are best made on the serum or the diluted plasma of the patient or animal according to the standard conditions suggested by Wallace and Wormall (1931), whereby satisfactory mixing of the reactants is obtained and agglutination of the red cells rarely occurs: where agglutination does occur, it is practically impossible to obtain a true measure of the amount of adhesion. With this technique there is also a very marked diminution in the number of cases of "non-specific" adhesion, *i.e.* adhesion with the serum or plasma of an individual who has not been infected with trypanosomes. In this form, or indeed in any form, the test is not ideal for use in the field,

and in view of the number of strains of trypanosomes required for satisfactory investigations and the amount of animal work involved, the test is essentially one for the laboratory. Thus, the observation made here that serum which has been preserved by the addition of phenol and then kept at the ordinary temperatures (23–26° C.) can be used a few days or even 3 weeks later for red-cell adhesion tests, appears to add to the value of the test. The amount of phenol used for this purpose should be sufficient to prevent appreciable bacterial decomposition, but a large excess of phenol should be avoided. A suitable amount is 1 volume of 2·5 per cent. phenol to 10 volumes of serum.

It should be pointed out that this test does not necessarily furnish evidence as to the results of drug treatment of trypanosomiasis, although tests carried out over a long period of time might have some value in this respect. A positive reaction indicates, however, "recent or actual infection with a trypanosome of the same group, but not necessarily identical with that employed as antigen in the test" (Duke and Wallace, 1930). Johnson and Lester (1928–9) report that the power to give platelet adhesion gradually disappears after a varying period following infection and treatment, and the suggestion is made by these authors that this test gives an indication of the acquired immunity, and that as this immunity is lost, re-infection may occur.

SUMMARY.

1. The results of further tests with the red-cell adhesion phenomenon in trypanosomiasis of man and other animals, described first by Duke and Wallace (1930) and later by Wallace and Wormall (1931), are discussed.

2. The conclusion is reached that the tests should always be made with several different strains of trypanosomes; in this way the number of negative results with undoubted cases of trypanosomiasis is decreased considerably. It is also advisable to have available stock adhesin sera (human or monkey) so that preliminary tests can be made to determine whether the trypanosomes in any animal are capable of giving adhesion. In doubtful cases of trypanosomiasis, treatment with trypanocidal drugs should be started. This treatment usually results in a marked improvement in the power of the serum or plasma of men and monkeys infected with trypanosomes to give red-cell adhesion.

3. If a suitable small amount of phenol is added to the serum of a trypanosomiasis patient, this serum can be kept at the ordinary temperatures for 3 weeks or more without appreciable loss of its power to give red-cell adhesion. In this way it is possible to carry out tests at a central laboratory on sera which have been collected at outlying stations.

4. Experiments have been carried out to determine the amounts of adhesin and complement (or "X factor") required for red-cell adhesion.

5. The adhesin is non-dialysable, and in this, and many other respects it behaves like a true immune body.

The author has pleasure in acknowledging the help which he has received during this investigation from Dr H. L. Duke, Director of the Human Trypanosomiasis Institute, and from Drs A. R. D. Adams and J. M. Wallace.

REFERENCES.

BROWN, H. C. and DAVIS, L. J. (1927). The adhesion phenomenon as an aid to the differentiation of leptospira. *Brit. J. Exp. Path.* **8**, 397.

BRUSSIN, A. M. and BELETZKY, W. K. (1925). Rieckenbergs Phänomen und dessen Anwendung in bezug auf Immunitätsvorgänge. *Cent. f. Bakt.* I. Abt. Orig. **96**, 32.

DAVIS, L. J. and BROWN, H. C. (1927). The adhesion phenomenon, a special serological reaction occurring in trypanosomiasis. *Trans. Soc. Trop. Med. and Hyg.* **21**, 113.

DUKE, H. L. (1930). The discovery of *T. rhodesiense* in man in the Uganda Protectorate. *Trans. Soc. Trop. Med. and Hyg.* **24**, 201.

DUKE, H. L. and WALLACE, J. M. (1930). "Red-cell adhesion" in trypanosomiasis of man and animals. *Parasitology*, **22**, 414.

JOHNSON, W. B. and LESTER, H. M. O. (1928–9). The value of the adhesion phenomenon in estimating wild game "trypanosome reservoirs" and as an aid to diagnosis in human trypanosomiasis. *West African Med. J.* **2**, 144.

KRITSCHEWSKY, J. L. and TSCHERIKOWER, R. S. (1925). Ueber Antikörper, die die Mikroorganismen mit Blutplättchen beladen (Thrombozytobarinen). *Z. Immunitätsforsch.* **42**, 131.

LEUPOLD, F. (1928). Untersuchungen über Rezidivstämme bei Trypanosomen mit Hilfe des Rieckenberg-Phänomens. *Z. Hyg. Infektionskr.* **109**, 144.

REGENDANZ, P. and JURUKOFF, B. (1930). Ueber das Adhäsionsphänomen der Trypanosomen. (Agglomeration, Blutplättchenbeladung, Leukozyten-Attachement.) *Z. Immunitätsforsch.* **66**, 32.

RIECKENBERG, P. (1917). Eine neue Immunitätsreaktion bei experimenteller Trypanosomen-Infektion; die Blutplättchenprobe. *Ibid.* **26**, 53.

SAUNDERS, G. F. T. (1931). The adhesion phenomenon in trypanosomiasis. *West African Med. J.* **5**, 28. Cited from *Trop. Dis. Bull.* 1932, **29**, 282.

WALLACE, J. M. and WORMALL, A. (1931). Red-cell adhesion in man and other animals. II. Some experiments on the mechanism of the reaction. *Parasitology*, **23**, 346.

(*MS. received for publication* 24. IX. 1932.—Ed.)

THE PROPHYLAXIS OF MALARIA BY DRUGS.

By C. STRICKLAND, M.A., M.D. (Cantab.),
Professor of Medical Entomology,
and D. N. ROY, M.B., D.T.M. (Cal.),
Assistant Professor of Medical Entomology, School of Tropical Medicine, Calcutta.

CONTENTS.

	PAGE
Introduction	77
Material and methods	78
Experiments with quinoplasmine: case records	78
Summary	80
Experiments with quinine: case records	81
Summary	82
Observations on the utility of plasmoquine	83
Observations on the utility of quinine salts	83
The species of malaria parasites concerned in relation to the foregoing observations	84
Do prophylactic drugs act on sporozoites?	84
References	86

Introduction.

The prophylaxis of plasmodial infections, by means of drugs in general, and by plasmoquine in particular, has been the subject of two reports to which we wish to refer: (1) by James, Nicol and Shute (1931), and (2) by Swellengrebel and de Buck (1931). The authors of report (1) obtained complete success by the use of 0·06 g. of plasmoquine before, and daily for 6 days after, the bite of infected mosquitoes, while the authors of report (2), giving 0·03 g. of the drug daily, had but poor success, notwithstanding Ottolenghi and Brotzu (1929), whom they cite, having reported that 0·02 g. daily with 0·25 g. of quinine was a good practical prophylactic.

James wrote: "the next step should be to ascertain whether the preparation (plasmoquine) is effective when taken once daily and if so what may be the smallest daily dose which will prevent malaria infection in persons who are bitten by infected mosquitoes twice or thrice weekly over a prolonged period." "This is, indeed a question of much consequence to the practical sanitarian who is called upon to apply the result of these investigations in the field, for he is faced with the difficulty that 6 cg. of plasmoquine given daily is a dangerously high dosage."

Before we had seen the results obtained by Swellengrebel and de Buck we had commenced an enquiry into the minimal dosage necessary to ensure

prophylaxis. We were especially anxious to determine the effect of the drugs on the entrance of sporozoites into the human body, and our procedure differed somewhat from that adopted by the above-named workers. As it turned out we obtained almost uniformly negative results, but our observations are here described, not only to show what procedure and dosages have proved useless, but also because of other considerations which we will discuss later.

Material and methods.

The mosquitoes used in our experiments were all *Anopheles stephensi* reared in the laboratory from larvae caught in the Calcutta area or from eggs laid by gravid females in the laboratory. The adults after they had hatched out were kept alive in cages on raisins and moistened lint. It has been found that *stephensi* readily bites man at all times when starved, hence our routine procedure was to withdraw the pot of raisins 6–8 hours before we wished to feed the mosquitoes and put it back after they had taken their blood meal; they were accordingly fed on the volunteers for the experiments at any time during the day or night as it suited our convenience.

The drugs that we used were "quinoplasmine," quinine bisulphate and quinine bihydrochloride.

The volunteers, after the "prophylactic" course, were not given any other medicine unless fever developed, when the blood was examined and if parasites were found the patient was put on to therapeutic quinine.

Experiments with quinoplasmine: case records.

Volunteer I.

Before infecting bites: Dosage representing			After infecting bites	
Plasmoquine	Quinine sulphate	Hours	Dosage	Time
g.	g.	*ca.*	g.	
0·01	0·3	24	As before	Soon
0·01	0·3	3		

Two mosquitoes, heavily infected[1] with *Plasmodium falciparum*, fed on the blood of this volunteer. The patient had a rigor on the 16th day after the bite, and malignant tertian rings were found in his blood; the symptoms were severe.

Volunteer II.

Before infecting bites: Dosage representing			After infecting bites
Plasmoquine	Quinine sulphate	Hours	
g.	g.	*ca.*	
0·02	0·6	6	Nil

[1] Examinations of the salivary glands of all mosquitoes were made after they had fed on the volunteers and the degree of infection was noted for each insect.

Three mosquitoes, heavily infected with *P. vivax*, bit this volunteer. The man had a rigor on the 13th day after the bite, the blood being examined for plasmodia next day and benign tertian rings found; the symptoms were mild.

Volunteer III.

Before infecting bites: Dosage representing			
Plasmoquine g.	Quinine sulphate g.	Hours *ca.*	After infecting bites
0·01	0·3	7	Nil
0·01	0·3	6	
0·01	0·3	5	

Three mosquitoes, heavily infected with *P. falciparum*, fed on this man. The patient had fever with a severe rigor on the 11th day after the bite; the symptoms being severe.

Volunteer IV.

Before infecting bites: Dosage representing			
Plasmoquine g.	Quinine sulphate g.	Hours *ca.*	After infecting bites
0·03	0·9	5½	Nil

Two mosquitoes, heavily infected with *P. falciparum*, fed on this volunteer. The patient had fever with a rigor on the 12th day after the bite; the symptoms were severe.

Volunteer V.

Before infecting bites: Dosage representing			
Plasmoquine g.	Quinine sulphate g.	Hours *ca.*	After infecting bites
0·01	0·3	30	Nil
0·01	0·3	26	
0·01	0·3	23	
0·01	0·3		
0·01	0·3		

Eight mosquitoes, moderately to very heavily infected with *P. vivax*, fed on this subject. The patient complained of chilliness, and slight fever on the 15th day after being bitten. No parasite could be detected either in thin or thick films. The patient improved without any treatment.

Volunteer I[2].

Before infecting bites: Dosage representing			
Plasmoquine g.	Quinine sulphate g.	Hours *ca.*	After infecting bites
0·02	0·6	30	Nil
0·02	0·6	26	
0·02	0·6	7	
0·02	0·6	2	

Five mosquitoes, mostly very heavily infected with *P. vivax*, fed on this man. On the 10th day after the feed the patient complained of chilliness and rigor and his symptoms were more severe than in the preceding case. His blood showed very scanty benign tertian rings in thick films (thin films negative). This man had previously volunteered as No. I and had suffered then from a malignant tertian infection.

Volunteer VII.

Before infecting bites: Dosage representing			
Plasmoquine g.	Quinine sulphate g.	Hours *ca.*	After infecting bites
0·02	0·6	31	Nil
0·02	0·6	28	
0·02	0·6	21	
0·02	0·6	7	
0·02	0·6	4	

Ten mosquitoes, all heavily infected with *P. vivax*, fed on this volunteer. The patient complained of chilliness and slight fever on the 19th day. On the 20th day his blood was examined and no parasite found.

Summary (see also Table I).

(1) Quinoplasmine given in various ways *before* infecting mosquito bites, did not prevent the development of malarial fever in seven volunteers, though in the last case (volunteer VII) in which a dosage representing 0·10 g. of plasmoquine and 3 g. (45 grains) of quinine was given within 31 hours of the mosquito bites, the incubation period was extended to 19 days, and the infection appeared to be modified.

(2) Quinoplasmine given *both before and after* the bites (volunteer I), *i.e.* 0·02 g. plasmoquine + 0·6 g. (10 grains) of quinine sulphate *before*, and 0·01 g. plasmoquine + 0·3 g. of quinine sulphate soon *after*, did not prevent the development of the infection.

(3) Quinoplasmine given soon *after* the infecting bites (volunteer I), in a dosage of 0·01 g. plasmoquine, and 0·3 g. quinine sulphate did not prevent the development of the infection.

Table I. *Recording the administration of prophylactic quinoplasmine to volunteers, none of who were saved from infection.*

Quinoplasmine representing a total dosage of			In the following no. of doses	The doses given at about the following no. of hours before the conveyance of infection	In Volunteer no.	Remarks
Plasmoquine g.	Quinine g.	Sulphate grains *ca.*				
0·02	0·60	9	2	24 and 3	I	He also received one other similar dose soon after the bite
0·02	0·60	9	1	6	II	
0·03	0·90	13½	3	7, 6 and 5	III	
0·03	0·90	13½	1	5½	IV	
0·05	1·50	22½	5	30, 26, 23, 4 and 2	V	
0·08	2·40	36	4	30, 26, 7 and 2	I[2]	
0·10	3·00	45	5	31, 28, 21, 7 and 4	VII	

EXPERIMENTS WITH QUININE: CASE RECORDS.

Volunteer VIII (*Quinine sulphate*).

Before infecting bites		After infecting bites
Dosage g.	Hours	
0·67 (=10 grains)	10	Nil
0·67	6	
0·67	4	

This man was bitten by two *P. vivax*-infected mosquitoes. He developed fever with rigor on the 13th day after the bite and *P. vivax* was found in his blood.

Volunteer IX (*Quinine bihydrochloride*).

Before infecting bites		After infecting bites
Dosage g.	Hours	
0·6 (=9 grains)	46½	Nil
0·6	27½	
0·6	25½	
0·6	22½	
0·6	3½	
0·6	½	

This man was bitten by ten *A. stephensi*, most of which were very heavily infected with *P. vivax*. 20 days after the infecting bites he had a rigor and *P. vivax* was found in his blood. He was a young man with chronic phthisis and gave no history of ever having had malaria, and his spleen was not palpable.

Volunteer X (*Quinine sulphate*).

Before infecting bites	After infecting bites		
	Dosage g.	Days	Hours
Nil	0·33 (=5 grains)	—	2
	0·33	1	
	0·33	2?	
	0·33	3	
	0·33	4	
	0·33	5	
	0·33	6?	
	0·33	7	
	0·33	8	
	0·33	9?	
	0·33	10	

This man was bitten by one *P. vivax*-infected mosquito. On the 2nd, 6th and 9th days he was given quinine to take in our absence and stated that it was taken. He developed fever and rigor on the 11th day, parasites being found 9 days after.

Volunteer XI (*Quinine sulphate*).

Before infecting bites		After infecting bites	
Dosage g.	Hours	Dosage g.	Days
0·33 (=5 grains)	2	0·33 (=5 grains)	1
		0·33	2
		0·33	3
		0·33	4
		0·33	5
		0·33	6
		0·33	7
		0·33	8
		0·33	9
		0·33	10
		Nil	(11–13)
		0·33	14

This man was bitten by nine *P. vivax*-infected mosquitoes. He did not develop malaria until the 40th day after being bitten. This case may represent another of those instances of lengthy incubation, observed by James and others. The volunteer stated that he had never previously had malaria.

Summary (see also Table II).

(1) *Quinine sulphate* given *before* the infecting bite in three doses of 10 grains each, within 10 hours, did not stop the infection (Vol. VIII).

(2) *Quinine sulphate* given in doses of 5 grains daily *before and after* the infecting bite was followed by development of infection after 40 days (Vol. XI).

(3) *Quinine sulphate* given *after* the infecting bites in doses of 5 grains a day for 10 days did not stop infection (Vol. X).

(4) *Quinine bihydrochloride* given in six doses of 9 grains (= 0·6 g.) each, before, and within 46½ hours of the infecting bites, was followed by development of infection, but the incubation period was extended to 20 days (Vol. IX).

Table II. *Recording the administration of prophylactic quinine to volunteers in all of whom infection developed.*

Total dosage	In the following no. of doses	The doses given at about the following no. of hours before	Volunteer No.	Remarks
Quinine sulphate				
2·0 g. =30 grains	3	10, 6 and 4	VIII	
0·33 g.= 5 „	1	2	XI	The same dose also given daily from the 1st to 10th days and 14th day after the bite
			X	0·33 g. (=5 grains) given from 1st to 10th days after the bite
Quinine bihydrochloride				
3·6 g.=54 grains	6	46, 27, 25, 22, 3½ and ½	IX	

OBSERVATIONS ON THE UTILITY OF PLASMOQUINE.

Results of other authors.

Russell (1931) showed that plasmoquine given only before inoculation of *Plasmodium cathemerium* was no protection against the development of the infection, but if given before and at least one dose after, the infection did not develop.

In the case of human malaria James, Nicol and Shute (1931) found that 0·06 g. of plasmoquine daily was sufficient protection, whereas Swellengrebel and de Buck with 0·03 g. daily had but poor success.

Our results.

We have found that no dosage of plasmoquine as we employed it was sufficient to protect a person from the development of infection. Of our dosages the largest total was 0·10 g. given within 31 hours before the infecting bites, while others varied from 0·02 g. to 0·10 g. given, with one exception only, before the infecting mosquito bites. The last-mentioned case does not confirm Russell's experience with *P. cathemerium* that the drug given at least once after conveyance of infection prevents it developing.

OBSERVATIONS ON THE UTILITY OF QUININE SALTS.

Results of other authors.

Yorke and Macfie (1924) have stated that quinine prophylaxis before experimental malarial infection is useless, and that its administration must be continued at least 10 days after, if success is to be achieved.

Russell (1931) working with *Plasmodium cathemerium* found that quinine given not only before, *but also after*, infection by inoculation did not protect canaries.

Swellengrebel and de Buck (1931) state that "no such results [like those of James with plasmoquine] were noted with prophylactic doses of quinine administered in the same way." Working with *P. vivax*, they found that the administration of about 10 grains a day after the infecting mosquito bites did not prevent the infection from developing, while 15 grains did so in one case though not in another. Indeed the authors distinguished between the action of quinine on those who had had malaria, from its action on those who had not had malaria previously, 15 grains of quinine protecting the former but not the latter.

Our results.

In our experiments, massive doses of quinine sulphate (30–45 grains), with or without the adjuvancy of plasmoquine, given only *before* (within 10 hours of an infecting mosquito bite) did not prevent development of infection, nor did 5 grains given daily after infecting bites. Massive doses of quinine bihydrochloride (54 grains) given only beforehand and within 48 hours (Volunteer IX) also did not prevent infection.

In volunteer XI the development of infection was delayed (40 days), and this may exemplify Swellengrebel and de Buck's contention that quinine may protect those who have had malaria before. Our case, however, differed from theirs in that we gave 5 grains instead of 15, and the subject had no previous history of malaria (which does not, however, count for much).

These observations will be disappointing to those who have habitually consumed 5 grains of quinine *per diem*, disappointing not only if they have never before had malaria (for it is commonly agreed that then this dosage will certainly not protect them from an attack), but also if they have already had an attack, for Swellengrebel and de Buck have only suggested, and this on the basis of but two observations, that such a large and impracticable daily dose as 15 grains is necessary to protect such persons from another attack.

The species of malaria parasites concerned in relation to the foregoing observations.

P. falciparum.

Quinoplasmine in an amount representing 0·03 g. of plasmoquine and 0·9 g. (about 15 grains) of quinine sulphate was given 7, 6 and 5 hours before the infecting mosquito bites were inflicted and development of malarial symptoms duly followed.

Quinine was not tested.

P. vivax.

Quinoplasmine did not prevent the development of this parasite even when a dosage represented as follows was employed:

(*a*) 0·08 g. of plasmoquine and 2·40 g. (total 36 grains) quinine sulphate within 30 hours before the infecting mosquito bites were inflicted.

(*b*) 0·10 g. of plasmoquine and 2·40 g. (total 45 grains) quinine sulphate within 31 hours (infection appeared to be modified in this case).

Quinine sulphate. 30 grains given within 10 hours *before* the conveyance of infection, and in another case 5 grains daily for 10 days *after* the infection did not suffice to retard the development of *P. vivax.*

Quinine bihydrochloride. 54 grains given between 48 hours and ½ hour before the infliction of the infecting mosquito bites did not stop the development of parasite.

Do prophylactic drugs act on sporozoites?

The original purpose of most of our experiments was to determine if possible the amount of protection afforded by drugs against the entry of sporozoites.

Yorke and Macfie (1924) stated that quinine prophylaxis before the infecting mosquito bite is useless, and that quinine has but little, if any, action on sporozoites.

Russell (1931) found that even after 7 days' administration of plasmoquine, protection was not afforded against infection by *Plasmodium cathemerium*

inoculated[1] 5 hours after the last dose of the drug (using the same dose of plasmoquine as had been found protective when given *after* inoculation). Russell also found that *quinine*, given before inoculation, afforded no protection.

James (1931) regards plasmoquine as of "such great importance because of its specific action on sporozoites," and Swellengrebel said "it would be of great practical importance in malaria prophylaxis to possess a drug which destroys the sporozoites on entering the human host," but the foregoing observers have not published any experimental evidence bearing on the subject. All their results can be interpreted as the result of the therapeutic action of the drug on the parasite after its entry into the human host. As Yorke and Macfie have said "the mechanism by which development of infection is prevented is similar to that by which a cure is effected," and Russell said that the protective action of plasmoquine is more therapeutic than prophylactic.

Therefore, the statements that "for destroying the sporozoites plasmoquine was the only known drug to be effective, and even plasmoquine for this purpose was not entirely satisfactory," and that "in the sexual forms plasmoquine acted excellently, and in doses much smaller than those effective against the sporozoites," appear to be unwarranted by any observations.

For those who sojourn in regions where malaria is endemic a continual dosage with a protective drug would of course be necessary in order to meet the continual possibility of being bitten by infected mosquitoes, so that it would not matter much whether the drug actually prevents the entry of the sporozoites or merely prevents the parasites from manifesting themselves thereafter. A drug fatal to the sporozoites would however be of especial value to those persons who have to enter endemic zones for short periods. Our experiments afford no evidence that sporozoites can be prevented from entering the human host.

Thus to refer back to but one case, where *P. falciparum* was the parasite concerned, 0·03 g. (about 15 grains) of quinine sulphate given from 7 to 5 hours before the infecting mosquito bite did not delay the development of infection, while with regard to *P. vivax* (*a*) 0·10 g. of plasmoquine and 3·00 g. (45 grains) of quinine sulphate given between 31 hours and up to 4 hours before the infecting mosquito bites did apparently no more than render the infection slight, (*b*) 0·08 g. of plasmoquine and 2·4 g. (36 grains) of quinine sulphate given within 30 and up to 2 hours before did not prevent the development of the infection, and (*c*) 54 grains of quinine bihydrochloride given between 48 and ½ hour before was likewise ineffective.

Then in twelve out of thirteen of Swellengrebel and de Buck's cases 0·03 g. of plasmoquine given 24 hours before the infecting bite did not stop the infection.

Such a number of observations and the massive amount of the drugs we used, seem to indicate that for all practical purposes neither plasmoquine,

[1] The trophozoites, not sporozoites.

quinine sulphate, nor quinine bihydrochloride have any effect on sporozoites. This conclusion coincides with Swellengrebel and de Buck's statement that "quinine leaves the subject to be protected at a great disadvantage by failing to act till the parasites have multiplied to a fairly strong number," and Yorke and Macfie's hypothesis that the host produces immune bodies in response to an antigen formation due to destruction of the parasite by a drug: the destruction of a relatively few parasites represented by the sporozoites would presumably not set up that process actively enough to prevent the development of infection. We consider that there is no prophylactic drug action against sporozoites demonstrated for malaria, and the effects hitherto observed must be considered to be a therapeutic action of the drugs on the parasites developing in man.

REFERENCES.

James, Nicol and Shute (1931). *Lancet*, **221**, 341–2.

Ottolenghi and Brotzu (1929). Effeti del trattamento con chinino o altri medicamenti nella prevenzione della malaria sperimentale. *Bollet. Soc. Ital. d. Biol. Sperim.* **4**, No. 2.

Russell, Paul F. (1931). Avian Malaria Studies. *Philippine J. of Sci.* **46**, 305, 347.

Swellengrebel and de Buck (1931). Prophylactic use of plasmoquine. *Proc. Konink. Akad. wetensch. Amsterdam*, **34**, No. 8.

Yorke, W. and Macfie, J. W. S. (1924). Observations on malaria made during treatment of general paralysis. *Trans. Roy. Soc. Trop. Med. and Hyg.* **18**, 13–33.

(*MS. received for publication* 3. x. 1932.—Ed.)

PLASMODIUM OVALE STEPHENS 1922.

By S. P. JAMES, W. D. NICOL and P. G. SHUTE.

(With Plate XI containing Figs. 1–14.)

In 1922 J. W. W. Stephens[1] reported that the morphological characters of the asexual forms of a malaria parasite which he had studied in the blood of a patient from East Africa were different from those of any usually accepted species, and he proposed to establish the parasite as a new species with the name *Plasmodium ovale*. In 1927, in collaboration with D. U. Owen[2], he confirmed and extended the description from the study of a case infected in Nigeria. In 1930 Warrington Yorke and D. U. Owen[3] studied another case from Nigeria and, by utilising this patient's blood in the practice of malaria-therapy, they were able to show that the peculiar morphological features of the asexual stages of the parasite remain constant when it is passed from person to person by direct blood inoculation.

In October, 1931, Prof. Warrington Yorke sent us a tube of citrated blood taken from a patient whom he had infected by direct inoculation with the blood of a patient from the Belgian Congo. We inoculated the blood intramuscularly into a patient who was awaiting treatment for general paralysis and had not previously been infected with any form of malaria. The patient developed the malarial attack after an incubation period of 6 days, and we were able to observe in this case that the morphological characters of the asexual parasites corresponded with the description of *P. ovale* given by previous workers. We infected several other patients by direct blood inoculation from this case and found that in all of them the course of the fever and the morphological characters of the parasite were the same. We then took up the problem of ascertaining whether the parasite could be passed through *Anopheles maculipennis* and, if that were so, whether the morphology of the parasite in patients infected by the bites of that mosquito would present the same characteristic features as in the original infecting case. We described this enquiry in a paper published in July, 1932[4], and about the same time showed preparations of the different stages of the parasite in the blood and in the mosquito at meetings of several scientific societies[5]. The enquiry proved that the peculiar morphological characters of *P. ovale*, the periodicity of its

[1] Stephens, J. W. W. (1922). A New Malaria Parasite of Man. *Ann. Trop. Med. and Parasitol.* **16**, 383.

[2] Stephens, J. W. W. and Owen, D. U. (1927). *Plasmodium ovale. Ibid.* **21**, 293.

[3] Yorke, W. and Owen, D. U. (1930). *Plasmodium ovale. Ibid.* **26**, 593.

[4] James, S. P., Nicol, W. D. and Shute, P. G. (1932). *Plasmodium ovale* Stephens: Passage of the Parasite through Mosquitoes and Successful Transmission by their Bites. *Ibid.* **26**, 139.

[5] Royal Society of Tropical Medicine and Hygiene, March 17th, 1932; Royal Society, June 22nd, 1932; British Medical Association Centenary Meeting, London, July, 1932.

asexual cycle in man and its clinical effects are not changed or modified after passage through the insect host. For this reason, as well as because we found that developing oöcysts of the parasite on the stomach of the mosquito can be easily distinguished from those of any of the three "classical" species previously recognised, there remained, in our opinion, no doubt that *P. ovale* must be regarded as a separate species.

Since the publication of the results of that enquiry several workers have written asking for further particulars than are contained in existing literature of the characters by which the parasite can be identified in stained blood films; others have drawn our attention to earlier findings of peculiar forms of the malaria parasite than that described by Stephens and have raised questions of priority of discovery and of nomenclature.

We have usually replied to enquirers in the first category by sending stained films of the parasite with an explanatory note describing our own procedure for identification. We hope that the coloured plate accompanying this paper, which Mr Jobling of the Wellcome Research Institution has kindly drawn from our preparations, will serve the same purpose as a film. The following is a revised statement of the contents of the explanatory note just mentioned:

(1) The parasite cannot be identified when the "thick film" or "thick drop" method of collecting blood is used. One must have thin films, spread with the usual precautions, and stained with Leishman's or with Giemsa's stain in such a way as to show clearly degenerative changes in infected red blood corpuscles as well as morphological details of the parasite. In studying the parasite for the purposes of this note we have employed the staining technique which we described in 1929[1].

(2) In deciding whether a particular parasite observed in a stained film resembles *ovale*, reliance cannot be placed on one's visual memory of the colour and shape of the pigment granules, the chromatin and the cytoplasm in the different known species of malaria parasite. Nor is it safe to depend upon one's own interpretation of a published description, however carefully worded it may be. For the descriptive terms commonly employed are not sufficiently precise to convey the same meaning to everyone. Our plan for overcoming these difficulties is to have at hand well-stained films of all the known species and to place them in turn under a microscope alongside the microscope which carries the blood film of the parasite to be identified. By visual comparison of the parasite to be identified with a known species of about the same stage of growth it can be decided at once whether, for example, the pigment has the same character and colour, whether the parasite is more or is less amoeboid and whether it causes the same change in the corpuscle in which it lies.

[1] James, S. P. (1929). A note on the Shute technique for staining malaria parasites with Leishman's stain and on the stippling in infected red blood corpuscles which it reveals. *Trans. Roy. Soc. Trop. Med. and Hyg.* **23**, 269.

(3) For correct identification it is also desirable to adopt, as a routine practice, a simple method of measuring the parasites and the red blood corpuscles containing them, and to compare these measurements with those of parasites and corpuscles in similar films of known species. It is not necessary that the measurements should state in *microns* the size of the parasites or corpuscles in the dry film: all that one needs to do is to ascertain how many divisions of an eyepiece micrometer cover the parasite to be identified (or the corpuscle in which it lies) as compared with the number which cover a known species at the same stage of growth. Using always the same magnification one might find, for example, that it is a general rule for the quartan parasite in the stage of complete "sporulation" to be covered exactly by four divisions of the micrometer scale. Now if it is found that the same stage of the parasite to be identified almost invariably covers five divisions, the indication is that one is not dealing with the quartan parasite, although the number of merozoites in the "sporulating" form may be the same.

(4) In searching for *P. ovale* in a stained film, as much attention should be given to changes in infected corpuscles as to the morphology of the parasite. It is hoped that the following notes may be helpful.

(1) When the parasite is in the stage of young rings.

At the youngest stage of growth of the parasite (rings of 2–2·5μ) the only reliable indication that the parasite may be *ovale* is the finding that nearly every corpuscle in which it lies is thickly studded with Schüffner's dots. This finding would make one think first of *P. vivax*, but it should be remembered that in an infection with *vivax*, however well stained the film may be, few corpuscles containing very young rings show Schüffner's dots and that, when they do, the dots are sparsely scattered through the cell and are like fine specks instead of being quite large and well defined. In our experience Schüffner's dots appear at an earlier stage of growth of the parasite and stain more readily and more deeply in infections with *ovale* than in *vivax* infections.

The parasite itself at this stage is not difficult to distinguish from rings of *P. falciparum*, as in these the cytoplasm stains a much lighter blue, is altogether less dense and solid and presents evidence of being actively amoeboid. Moreover, the chromatin in young rings of *falciparum* is usually in the form of a tiny round dot or perhaps two dots close together. Young rings of *ovale* are darker in colour, more solid and have a sharp well-stained outline; they are occasionally pear-shaped but never show further degrees of amoeboid activity. There is a relatively large mass of chromatin of irregular shape; the appearance is hardly ever that of the "bird's eye" form commonly seen in infections with other parasites. In less degree the same remarks apply to differences between the youngest ring forms of *ovale* and of *vivax*; the differences can be detected by visual comparison of typical preparations but may not be clear from a written description. On the other hand it is not possible

to detect, even by visual comparison, noteworthy differences between some young rings of *P. ovale* and some young rings of the quartan parasite *P. malariae*.

On the whole as regards identification of *ovale* at this stage of growth, unless one finds that nearly 100 per cent. of infected corpuscles are thickly studded with Schüffner's dots, it is wise to pass on to the examination of more advanced stages of growth.

(2) When the parasite is half-grown (with a single nucleus not yet beginning to divide).

There are several features on which one can rely for the identification of *ovale* at this stage of growth. Observing first the character of infected corpuscles one would find that they are larger than normal cells. If it had been found that a normal cell, on an average, is covered by five divisions of the eyepiece micrometer it would be found that an infected cell is covered by at least six divisions or, if the cell were not round, that its long diameter is covered by eight divisions, its short by four or five. Next it would be noted that the cell is thickly studded with Schüffner's dots deeply stained and well defined. These observations would exclude *P. malariae* and *P. falciparum* but not *P. vivax*. Continuing to study the characters of infected corpuscles it might be found that a large proportion of them were of peculiar shape and appearance. What would strike the observer most would be that the margin of many infected cells, instead of appearing as a well-defined line, would seem to have been drawn out into more or less ragged points (Fig. 6). Even supposing that isolated examples of this fringed or "fimbriated" appearance of infected corpuscles might be found by long search of films of *vivax* infection, it is such a frequent feature of infections with *P. ovale* that it is a character by which this infection can be identified. When the points which form the fringe are examined it will be noted that this part of the margin of the corpuscle has become so decolorised and transparent that some of the points seem to be composed only of deeply stained Schüffner's dots which appear, for this reason, to extend beyond the outline of the corpuscle itself.

About the parasite itself at this stage of growth, its resemblance to the quartan parasite, *P. malariae*, is rather striking, especially if reliance is placed on one's visual memory of what a quartan parasite looks like rather than upon the safer plan of making a comparative examination of typical films. If one adopts this plan and uses at the same time an eyepiece micrometer it will be found that the quartan parasite at the same stage of growth is considerably smaller and that the pigment which it contains is darker in colour and much more conspicuous.

(3) WHEN THE NUCLEUS OF THE PARASITE HAS DIVIDED INTO FOUR OR MORE PARTS.

This is the stage of growth at which there is least difficulty in distinguishing *ovale* from other species, for by this time most corpuscles containing the parasite show peculiarities of shape and other degenerative changes which are not seen (or are only very rarely seen) in infections with other species. A striking feature is that about 25 per cent. of corpuscles containing this stage of the parasite are definitely of oval shape. The picture seen under the microscope is a round parasite in the centre of an oval cell[1]. In examining blood films from a case with many parasites one encounters that picture so frequently that one cannot fail to be struck by its diagnostic significance. The oval corpuscle, in its long diameter, may be covered by from seven to ten divisions of the eyepiece micrometer, but in its short diameter it is covered almost invariably by four, which is also the diameter of the circular parasite at this stage of its growth. Occasionally in examining a film of *P. vivax* one may find infected corpuscles of oval shape (the "Rugby football" form), but in those examples the parasite is also of oval shape and nearly fills the cell. Another diagnostic feature of *ovale* at this stage is that the infected corpuscles may have part of their outline fringed, as was described for cells containing the half-grown parasite, and may present the characteristic appearance of Schüffner's dots extending beyond the contour of the corpuscle (Figs. 5 and 7).

The parasite itself at the stage when it contains four or more separate masses of chromatin is regularly round in shape and usually measures 6·2 μ. Thus it is about the same size as the quartan parasite at that stage and is definitely smaller than *P. vivax*. It can be distinguished from the quartan parasite by the appearance of the pigment which is lighter in colour and less coarse.

(4) IN THE STAGE OF COMPLETE SEPARATION OF THE MEROZOITES (SO-CALLED "SPORULATION").

The picture at this stage is that of eight merozoites arranged round a central block of pigment in a corpuscle heavily studded with Schüffner's dots (Fig. 4). The small number of merozoites distinguishes the parasite from *P. vivax* and the Schüffner dotting of the corpuscle distinguishes it from *P. malariae*.

(5) THE SEXUAL FORMS OF THE PARASITE.

It will be seen from Figs. 8 and 9 that these forms can be readily distinguished from those of *P. malariae* by the characters of the corpuscles in which they lie. It is not so easy to distinguish them from the sexual forms of *P. vivax* especially as they are seldom or never contained in oval corpuscles. By visual comparison it can be seen that the pigment has a different colour and character, but these differences are not easy to describe.

[1] Some workers who have written to us have thought that the name *P. ovale* indicates that the parasite is often oval in shape. This is not so.

(6) Stages of development in the mosquito.

Although this note is concerned primarily with the identification of the parasite in blood films, it will not be out of place to describe the points on which we rely for recognising it in the mosquito. The four important stages of the parasite in the insect host are illustrated in Figs. 10–14. The "exflagellating" stage and oökinete stage ("travelling vermicule") are smaller than in other species of the malaria parasite but present no particular distinguishing characters. The oöcyst stage, however, is characteristic and can be identified without difficulty by noting the character and arrangement of the pigment in forms not older than the stage of growth reached in 72 hours at 25° C. This may be described for the different species as follows:

P. vivax: The grains of pigment are numerous (more than thirty or forty) and are of a lighter brown colour than in any other species. They are thin rods and specks scattered throughout the parasite in no definite pattern.

P. malariae: The grains of pigment are numerous but are larger, thicker and much darker in colour than those of *P. vivax*. In shape they are more like cocci than like rods or specks and they tend to be bunched together in a clump at one or other part of the oöcyst. A row or circle of grains may extend from the clump round the contour of the zygote.

P. ovale: The grains of pigment are more like those of *P. malariae* than of *P. vivax*. They are always arranged in one or other of several definite patterns of which the most common is two rows of dots crossing each other at right angles (Figs. 12 and 13). This pattern is maintained up to the eighth or ninth day of growth.

P. falciparum: The contour of the oöcyst is remarkably well defined. The grains of pigment are few in number (about ten or twelve), large in size and black in colour. They are arranged very characteristically as a streak across the oöcyst or as a half-circle.

The sporozoites of *P. ovale* as they appear in a stained smear from the salivary glands of *A. maculipennis* are illustrated in Fig. 14. A striking character is their small size. Unfortunately we have no preparation of sporozoites of the quartan parasite but we have made many measurements of stained sporozoites of *ovale* in comparison with similar preparations of sporozoites of *falciparum* and *vivax*. Our comparative figures of length for these three species are 9:11:13. We have not attempted to measure sporozoites in the fresh state so the figures are useful only for purposes of comparison.

(7) General remarks.

In several letters which we have received it is said that the writer has observed in a case of infection with *P. vivax* peculiar forms of the parasite which seem to agree in morphological characters with published descriptions of one or other stage of *P. ovale*. The reference in these letters is to a strain of *P. vivax* which, in the practice of malariatherapy, has been passed a number

of times from man to man by direct blood inoculation without the intervention of the mosquito. The literature relating to induced malaria also contains some references to the subject. For example, Missiroli and Siniscalchi (1929)[1], studied the morphological changes in a strain of *P. vivax* up to its sixth direct passage. By examining blood films taken from all the cases inoculated with this strain they found in some films parasites which were typical of *P. vivax*, in other films parasites resembling the *tenue* type of *P. falciparum*, in others parasites resembling some stages of *P. ovale*. They recall that observations of the same kind were made by Mühlens and Kirschbaum[2] as regards forms resembling *tenue* and by Plehn[3] as regards forms resembling *ovale*. Occasionally the same observation has been recorded in particular cases of naturally acquired benign tertian malaria. In 1920 one of us[4], describing some unusual findings in cases of malaria contracted during the war, reported as follows: "In old-standing cases some of the full-grown benign tertian forms in red cells which were deeply stippled with Schüffner's dots, were not larger than a normal blood corpuscle, and formed rosettes consisting of only ten to twelve young parasites. Moreover, the red cell in which they lay, although it was profusely spotted with Schüffner's dots, was only slightly enlarged." Catanei (1923)[5] has summarised all the available information contained in the publications of fifteen observers who had described "atypical forms" of the different "classical" species of the malaria parasite. Thus it is evident that in searching for cases of malaria caused by *P. ovale* one must keep in mind the possibility that a particular parasite encountered in a blood film may be only an abnormal form of one of the three "classical" species. As Wenyon[6] has said "it cannot be too forcibly emphasised that the parasites of malaria, like all living organisms, are liable to appear occasionally in abnormal form." For this reason one must not be content to identify *ovale* from the appearance of only one stage of the parasite; one must continue to examine the film and, if possible, films taken on subsequent days, until all the stages described in this note have been passed under review and have been observed to agree with the description.

We must now mention briefly the questions of priority of discovery and of nomenclature to which several correspondents have drawn our attention. The reported findings of only two observers seem to be of essential importance in this connection, namely that of Craig in 1900 and that of Ahmed Emin in 1914.

[1] Missiroli, A. and Siniscalchi, R. (1929). Sulle modificazioni morfologiche e biologiche dei parassiti malarigeni nei trapianti interumani. *Riv. di Malariol.* (Rome), **8**, 251.

[2] Mühlens, P. and Kirschbaum, W. (1921). Parasitologische und klinische Beobachtungen bei künstlichen Malaria- und Rekurrensübertragungen. *Z. Hyg. u. Infektionskr.* **94**, 1.

[3] Plehn, A. (1925). Beobachtungen bei Paralytikermalaria. *Arch. f. Schiffs- u. Tropenhyg.* **29**, 2, 56.

[4] James, S. P. (1920). *Malaria at Home and Abroad*, p. 169.

[5] Catanei, A. (1923). Essai Critique sur la Morphologie des Parasites du Paludisme. *Arch. Inst. Pasteur d'Algérie*, **1**, 10.

[6] Wenyon, C. M. (1926). *Protozoology*, II, 953.

In the report for 1900 of the Surgeon General of the United States Army, Colonel C. F. Craig described from fresh preparations a malaria parasite which he found in the blood of soldiers who had returned from service in the Philippine Islands and were suffering from fever with tertian paroxysms indistinguishable clinically from those caused by *P. vivax*. In 1914 he described the parasite again from preparations coloured with Wright's stain[1]. He expressed the view that the morphology of the parasite was quite different from that of *P. vivax* and, although his description of it differs in some minor respects from present-day descriptions of *P. ovale*, it is reasonable to suppose that this was the parasite which he had found. At any rate he is of this opinion. In September, 1932, following a correspondence which he had had with Prof. Yorke on the subject, we sent him some stained films of *P. ovale* for examination. On September 30th he replied as follows: "I have studied your preparations carefully and can say without any question and without any doubt at all in my mind that this parasite is identical with the one that I described in 1900."

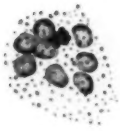
The question whether Dr Ahmed Emin (1914)[2] also found and described *P. ovale* before the publication of Prof. Stephens' paper, is not so easy to answer. Emin's paper contains an account of the parasites observed in cases of malaria occurring among pilgrims detained in hospital at Camaran *en route* for Mecca. In 90 per cent. of the cases the parasite found was *P. vivax*; in the remainder it was *P. falciparum*. There were cases of mixed infection with both those parasites but no case of infection with *P. malariae* was seen. In six patients a type of parasite was found which Dr Emin thought had not been previously described. The parasite was present in great numbers and the patients were severely ill. It is evident from Dr Emin's paper and from the illustrations which accompanied it that the blood films from these patients contained either a mixed infection with two species (of which one was certainly *P. falciparum*) or that they were films from severe cases of *P. falciparum* in which the peripheral blood contained half-grown and segmenting stages of this parasite as well as many ring forms. That the films contained *P. falciparum* cannot be doubted by anyone who reads Dr Emin's description or examines the illustrations numbered 1 to 8 of his plate. His own explanatory note of these figures was: "Toutes ces formes sont identiques à celles qu'on trouve dans la tierce maligne." He added that four or five of these young forms were often present in the same corpuscle, that they were not larger than rings of the tropical parasite, and that they showed very active amoeboid movements. Thus the only difficulty in interpreting his paper and illustrations is concerned with the forms illustrated in his Figs. 9–26, which he described as being more advanced stages of development of the young ring forms just mentioned. Probably that view was correct but, unfortunately, in describing them Dr Emin wrote that they were "analogues à celles de la tierce bénigne, avec cette différence que les rosaces renferment moins d'éléments et que les

[1] Craig, C. F. (1914). New varieties and species of malaria plasmodia. *J. Parasitol.* Urbana, **1**, 85.
[2] Emin, A. (1914). Une variété nouvelle du parasite de Laveran. *Bull. Soc. Path. Exot.* **7**, 385.

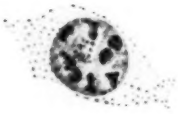
1

2

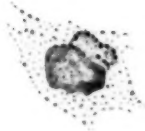
3



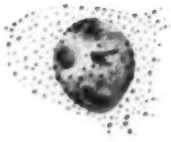
4



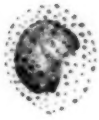
5



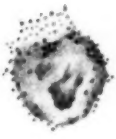
6



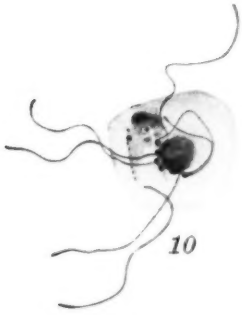
7



8

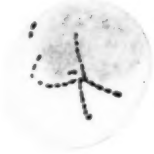


9



10

11



12

13

14

UNIV. OF MICH.

B. Jobling del.

Plasmodium ovale Stephens 1922.

globules ne sont ni décolorés ni augmentés de volume." Perhaps he would not have likened them to benign tertian parasites if he had been familiar with the appearance of advanced and segmenting forms of the malignant tertian parasite which are occasionally present in the peripheral blood of severe cases such as those with which his paper was concerned. For, in addition to the fact that many of the corpuscles containing these developing and segmenting forms were marked with Maurer's spots, Prof. Ziemann[1], who examined some of the films sent to him for the Pasteur Institute, Paris, by Prof. Marchoux, said that the parasites themselves bore no resemblance to forms of *P. vivax*. He classed them as examples of a pernicious type of the tropical parasite and proposed the name *P. camarense* Emin.

The origin of the prevalent opinion that *P. ovale* is the same parasite as that described by Emin is not clear. It is not the opinion of observers who have actually examined blood films of both parasites. One of us recently discussed the subject with Prof. Marchoux who examined Emin's films before they were sent to Prof. Ziemann. After studying our preparations of *P. ovale*, Prof. Marchoux said that Emin's parasites were of a quite different type, and that from now onwards (whatever opinions may have been expressed in the past) no question of their being the same parasite as *ovale* could arise. Colonel C. F. Craig and others, whose former opinion of the identity of the two parasites was based on published descriptions only, have now, after studying our preparations of *P. ovale*, written to the same effect. It should be noted also that Stephens and Owen (1927)[2] stated categorically that they were unable to identify *P. ovale* with Emin's parasite.

For these reasons we should like to suggest general acceptance of the view that there is in reality no disputable question concerning the nomenclature of *P. ovale*. Its name stands because in the first place Craig did not name the parasite which he described in 1900 and, in the second, because it is not the parasite which Emin described in 1914.

EXPLANATION OF PLATE XI.

Plasmodium ovale Stephens 1922.

Fig. 1. Ring form.
Fig. 2. Very rare form somewhat resembling a "band form" of *P. malariae*.
Fig. 3. Two schizonts in the same corpuscle.
Fig. 4. Stage of complete separation into merozoites.
Figs. 5, 6 and 7. Examples of stages 2 and 3 described in the text.
Fig. 8. Female gametocyte.
Fig. 9. Male gametocyte.
Fig. 10. Exflagellating male form.
Fig. 11. Oökinete.
Figs. 12 and 13. Oöcysts from the stomach wall of the mosquito.
Fig. 14. Sporozoites from the salivary glands of the mosquito.

[1] Ziemann, H. (1915). Ueber eigenartige Malariaparasitenformen. *Centralbl. Bakt.* 1 Abt. **76**, 384.
[2] See p. 87, note 2.

(*MS. received for publication* 18. XI. 1932.—Ed.)

THE ACTION OF PLASMOQUINE ON MOSQUITO-INDUCED MALARIA OF BIRDS[1].

By P. TATE and M. VINCENT.

From the Molteno Institute for Research in Parasitology, University of Cambridge.

The action of the synthetic quinoline compound plasmoquine on avian malaria was first investigated by Roehl (1926), who found that it is very effective in preventing the appearance of malaria parasites in the blood of birds infected by direct blood inoculation. The action of plasmoquine on the bird malarias was further studied by Hegner and Manwell (1927), Manwell (1930, 1932), Fourneau and co-workers (1930), Russell (1931) and others. All these workers studied only malaria induced by direct blood inoculation and no results have as yet been published on the action of this compound on avian malaria induced by mosquitoes, although it is known that in malaria of man (benign tertian) cases infected by blood inoculation are more amenable to quinine treatment than are cases infected by mosquitoes.

Recently James, Nicol and Shute (1931) investigated the prophylactic action of plasmoquine on mosquito-induced malaria of man and found that 0·02 g. thrice daily prevented mosquito-borne malarial infections in human volunteers. In a later paper by James (1932) a chart giving the later history of the prophylactic experiment shows that half the volunteers developed malaria six months after infection. Swellengrebel and de Buck (1931) and Swellengrebel (1932) found that half the daily dose of plasmoquine given by James did not prevent mosquito-induced malaria in volunteers.

Recently we have compared the action of plasmoquine in bird malaria induced by direct blood inoculation and by the bites of infected mosquitoes (*Culex pipiens* L.). In some preliminary experiments we used a German strain of *Plasmodium relictum* Grassi and Feletti kindly provided by the late Dr Roehl of Elberfeld; this we call strain G; but for the main experiments we used a strain of *P. relictum* obtained through the kindness of Prof. Sergent of Algiers, which gives a higher infection rate in mosquitoes and is more virulent in canaries than the German strain; this we call strain A.

The birds used were hen canaries of the German roller variety. Blood inoculations were made intramuscularly using infected blood diluted with citrate-saline solution. In mosquito-induced malaria experiments the control birds (untreated) and the birds treated with plasmoquine were bitten by

[1] This work was done on behalf of the Chemotherapy Committee of the Medical Research Council, London.

mosquitoes from the same lots. The number of mosquitoes given as biting the experimental birds represents only those which were proved by dissection after biting to have sporozoites in the salivary glands. The plasmoquine solution was made up freshly from "Plasmoquine simplex" of Bayer and was given orally using the technique described by Roehl (1926). The doses given are calculated in mg. of compound per 20 g. of body weight of bird. The standard dose is 0·5 c.c. of solution for a bird of 20 g. weight, and the volume of solution is varied in proportion to the weight of the birds. According to Roehl the maximum tolerated dose of plasmoquine is 0·64 mg., but we found that few birds tolerate this dose repeatedly, and we consider that 0·32 mg. is the highest safe dose.

In our experiments we tested the possible prophylactic action of plasmoquine given in large doses (0·32 mg.) for varying periods before and after infection by mosquitoes. Birds which did not show parasites in the blood for long periods were tested for latent infections by reinoculation, a method which was introduced by Sergent (1920).

EXPERIMENT I.

Plasmoquine given after mosquito infection with malaria strain G.

Bird 1, bitten by one infected mosquito and immediately given 0·32 mg. plasmoquine and the same dose 24 hours later. Parasites appeared in blood on 7th day.

Bird 2, control, bitten by one infected mosquito but did not develop malaria.

Bird 3, bitten by five infected mosquitoes and given 0·32 mg. plasmoquine immediately after and the same dose 24 hours later. Parasites appeared in blood on 9th day.

Bird 4, control, bitten by two infected mosquitoes. Parasites appeared in blood on 9th day.

Thus, in this experiment two doses of plasmoquine following mosquito infection had no effect.

EXPERIMENT II.

Plasmoquine given before and after mosquito infection with malaria strain G.

Bird 5 was given 0·32 mg. plasmoquine at 5.30 p.m. 26. vii, similar doses at 11 a.m. and 5.30 p.m. 27. vii; was bitten by one infected mosquito during the night 27–28. vii; and received another dose of plasmoquine at 11 a.m. 28. vii. Treatment was discontinued owing to the toxicity of the drug. Blood films remained negative until 66th day, when the bird was successfully reinoculated, showing that there was no latent infection.

Bird 6 was given 0·32 mg. plasmoquine at 5.30 p.m. 26. vii, the same dose at 11 a.m. 27. vii; was bitten by one infected mosquito about 5.30 p.m.;

and was given 0·32 mg. plasmoquine immediately after and at 11 a.m. 28. vii. Toxic symptoms prevented further treatment. The blood remained negative for 66 days when the bird was successfully reinoculated.

Bird 7, control, bitten by one infected mosquito 27. vii. Parasites appeared in blood on 12th day.

The failure of birds 5 and 6 to develop malaria may be due to the action of plasmoquine in doses on the level of toxicity. However, as we have not obtained a 100 per cent. infection in control birds bitten by only one or two lightly infected mosquitoes the result with birds 5 and 6 affords no definite proof of the prophylactic action of the drug.

Experiment III.

Plasmoquine given before mosquito infection, and both before and after infection, with malaria strain A.

Details of this experiment are given in Table I. The figures in the second column represent the number of mosquitoes with sporozoites in the salivary glands which bit each bird. The doses of plasmoquine are shown as follows: $3 \times 0{\cdot}32$ means that three doses each of 0·32 mg. were given at 24-hour intervals. On the day of infection the birds were bitten about 2 hours after receiving the plasmoquine.

Table I.

Bird	Number of infective mosquitoes	Plasmoquine before infection	Plasmoquine after infection	Day parasites appeared in blood
8	1	$3 \times 0{\cdot}32$	—	7
9	4	$3 \times 0{\cdot}32$	$1 \times 0{\cdot}32$	6
10	2	$3 \times 0{\cdot}32$	$1 \times 0{\cdot}32$	8
11	2	$3 \times 0{\cdot}32$	—	Negative to 59th*
12	1	Control	—	6
13	1	Control	—	7

* Then successfully reinoculated.

This experiment shows that plasmoquine given in large doses for three days before infection, or for three days before and one after, had no prophylactic action in three out of the four birds treated. The one bird which has not yet shown parasites had no plasmoquine after infection, while the two birds which received additional doses the day after infection developed malaria as soon as the control birds.

Experiment IV.

Plasmoquine given after infection with malaria strain A. Comparison of its action in blood-inoculated and mosquito-infected birds.

Parallel series of birds were infected by (*a*) direct blood inoculation, and (*b*) bites of infected mosquitoes. Both series were infected as nearly as possible

at the same time and were treated with plasmoquine, the first dose being given 4 hours after infection and subsequent doses at 24-hour intervals. Details are given in Table II (dosage as in Table I).

Table II.

Bird	Mode of infection	Plasmoquine	Day parasites appeared in blood
14	Blood inoculation	Control	6
15	,,	6 × 0·32	Negative to 55th*
16	,,	6 × 0·32	Negative to 55th
17	,,	1 × 0·32	10
18	,,	1 × 0·32	13
19	,,	6 × 0·02	21
20	,,	6 × 0·02	Negative to 55th
21	,,	Control	6
22	Mosquitoes 2	Control	7
23	,, 3	6 × 0·32	8
24	,, 2	3 × 0·32	9
25	,, 3	1 × 0·32	6
26	,, 3	1 × 0·32	8
27	,, 1	Control	8

* Then successfully reinoculated.

The results show definitely that while in blood-inoculated malaria, plasmoquine, even in very small doses of 0·02 mg., causes great delay in the appearance of parasites in the blood, in mosquito-infected cases it has no effect even in doses sixteen times larger.

EXPERIMENT V.

Plasmoquine given for varying periods after mosquito infection with malaria strain A.

The birds received a dose of plasmoquine 4 hours after infection, and similar doses for varying periods on the following days. Birds 28 and 31 received two doses, one at 11 a.m. and one at 5 p.m., on the day after infection (see Table III) and one dose daily subsequently. For explanation see previous tables.

Table III.

Bird	Number of infective mosquitoes	Plasmoquine	Day parasites appeared in blood
28	3	7 × 0·32	9
29	1	4 × 0·32	Died 4th day
30	1	Control	7
31	2	4 × 0·32	8
32	1	6 × 0·32	11
33	1	Control	7
34	2	11 × 0·32	16
35	3	6 × 0·32	8

This experiment shows that plasmoquine given after mosquito infection, even if continued for 6 days, has no significant effect on the time of appearance of parasites in the blood; and, although longer treatment prevents the appearance of parasites while it is continued, they appear in the blood soon after treatment terminates (bird 34).

In comparing the efficacy of a drug in blood-inoculated and mosquito-borne avian malaria the reliability of the two methods of infection must be considered. In the mosquito-infection experiments the proportion of untreated birds (controls) which did not show parasites was 1 in 9, while with the treated birds it was 3 in 17, so that the difference between the treated and untreated birds gives no indication of the prophylactic action of plasmoquine. On the other hand, we have found that with direct blood inoculation, with acutely infected blood, the failures to develop malaria are less than 1 per cent. out of many hundreds of untreated birds. Consequently, the failure of the blood-inoculated birds treated with plasmoquine to develop malaria may be accepted as proof of the action of the drug.

In the mosquito-infected birds treated with plasmoquine there was no marked difference in the severity of the malaria attacks compared with those of the control birds, except in the case of bird 34 which after 11 days' treatment with plasmoquine had a very slight attack.

The difference in the effect of plasmoquine on blood-inoculated and mosquito-induced malaria may be due either to the resistance of the sporozoites to the action of plasmoquine or to their rapid penetration into cells where they are protected.

Summary.

The experiments described show that plasmoquine is very effective in preventing or delaying attacks of malaria in birds infected with *P. relictum* by direct blood inoculation, but has little or no prophylactic action in birds infected by the bites of mosquitoes.

In the mosquito-induced malaria parasites may appear in the blood 3 or 4 days after the cessation of plasmoquine treatment and there is no appreciable delay as compared with untreated control birds.

REFERENCES.

Fourneau, E., Trefouel, M. et Mme, Stefanopoulo, G., Benoit, G., De Lestrange, Y. et Melville, Kenneth J. (1930). Contribution à la chimiothérapie du paludisme. Essais sur la malaria des canaris. *Ann. Inst. Pasteur*, **44**, 503–33.

Hegner, R. and Manwell, R. D. (1927). The effects of plasmochin on bird malaria. *Amer. J. Trop. Med.* **7**, 279–85.

James, S. P. (1932). Discussion. Synthetic antimalarial remedies and quinine. *Trans. Roy. Soc. Trop. Med. and Hyg.* **26**, 105–9.

James, S. P., Nicol, W. D. and Shute, P. G. (1931). On the prevention of malaria with plasmoquine. *Lancet*, ii, 341.

Manwell, R. D. (1930). Further studies on the effect of quinine and plasmochin on the avian malarias. *Amer. J. Trop. Med.* **10**, 379–405.

—— (1932). Quinine and plasmochin therapy in *Plasmodium rouxi* infections, with further notes on the effects of these drugs on the other avian malarias. *Ibid.* **12**, 123–47.

Roehl, W. (1926). Die Wirkung des Plasmochins auf die Vogelmalaria. *Arch. f. Schiffs- u. Tropenhyg.* Beiheft 3, **30**, 11–18.

RUSSELL, P. F. (1931). Avian malaria studies. I. Prophylactic plasmochin in inoculated avian malaria. *Philippine J. of Sci.* **46**, 305–43. II. Prophylactic plasmochin versus prophylactic quinine in inoculated avian malaria. *Ibid.* **46**, 357–61.

SERGENT, ET. (1920). Le diagnostic de l'infection latente dans le paludisme des oiseaux. *C.R. Soc. Biol.* **83**, 1063–4.

SWELLENGREBEL, N. H. (1932). Discussion. Synthetic antimalarial remedies and quinine. *Trans. Roy. Soc. Trop. Med. and Hyg.* **26**, 114–15.

SWELLENGREBEL, N. H. and DE BUCK, A. (1931). Prophylactic use of plasmoquine in a dosage warranting reasonable safety for routine treatment. *Proc. Roy. Acad. Sci. Amsterdam*, **34**, 1216–20.

(*MS. received for publication* 15. XII. 1932.—Ed.)

COCCIDIAL INFECTIONS IN THE BADGER *MELES TAXUS* L.

By A. KOTLÁN and L. POSPESCH.

Laboratory of Parasitology, Royal Hungarian Veterinary College, Budapest.

(With Plate XII, containing Figs. 1–16.)

In continuation of our investigations[1] on the relationship between the various Coccidia living in man and carnivorous animals (dog, cat, fox, etc.), we have had the opportunity of examining the intestines of several badgers, *Meles taxus*, which died in the Zoological Gardens, Budapest. These examinations revealed in a number of cases the occurrence of Coccidia belonging to both Eimeridae and Diplosporidae. In order to eliminate the possibility of these coccidial infections having been derived from other animals (fox, dog, cat), kept in close proximity with the badgers, it was found necessary to determine whether such infections also occur in badgers living under natural conditions. With the kind help of Prof. Raitsits, head of the ambulatory clinics of our College, we succeeded in obtaining a young specimen of *Meles taxus*, which not only harboured Coccidia, but subsequently served as an experimental animal for various cross-infections.

Hitherto Coccidia from the badger have not been recorded, but we have found at least two different species which may develop in the badger's intestines, one being an *Eimeria* (apparently a species characteristic of this host only), whilst the other, belonging to the Diplosporidae, exhibits morphological features closely resembling a diplosporid form of the dog or cat.

During our investigations the material at hand was repeatedly examined with the following results:

Specimen No. I. Young adult, from the Zoological Gardens. Oöcysts of *Eimeria* and *Lucetina* were present, the latter in moderate numbers only. Measurements:

Eimeria		*Lucetina*	
17 × 15 μ	21 × 15 μ	26 × 20 μ	30 × 26 μ
19 × 14 μ	22 × 14 μ	27 × 22 μ	30 × 27 μ
20 × 14 μ	23 × 15 μ	28 × 23 μ	31 × 24 μ
20 × 15 μ	24 × 15 μ	29 × 24 μ	34 × 27 μ
Range: 17–24 × 14–15 μ		Range: 26–34 × 20–27 μ	

Specimen No. II. Adult from the Zoological Gardens. No Coccidia were found.

[1] These investigations constitute a part of our studies on Coccidia and are being conducted with the financial support of the Hungarian Board of Natural Science.

Specimen No. III. Adult, from the Zoological Gardens. Oöcysts of both *Eimeria* and *Lucetina* were found. Measurements:

Eimeria		*Lucetina*	
18 × 15 μ	21 × 16 μ	26 × 19 μ	29 × 22 μ
19 × 14 μ	21 × 14 μ	26 × 20 μ	30 × 23 μ
20 × 14 μ	22 × 14 μ	27 × 21 μ	31 × 21 μ
21 × 13 μ	23 × 17 μ	28 × 22 μ	31 × 23 μ
Range: 18–23 × 13–17 μ		Range: 26–31 × 19–23 μ	

Specimen No. IV. Young adult, captured wild. Diplosporid oöcysts only were found. Measurements:

Lucetina	
26 × 20 μ	29 × 21 μ
26 × 21 μ	31 × 22 μ
27 × 21 μ	31 × 23 μ
28 × 24 μ	33 × 26 μ
Range: 26–33 × 20–26 μ	

The foregoing data show that out of three badgers which died at the Zoological Gardens, two were found infected with both *Eimeria* and *Lucetina*, while the third was free from coccidial infection.

THE EIMERID COCCIDIUM (Figs. 1–4).

A young specimen of *Meles taxus* which, after having been captured, was brought to the laboratory, showed an infection with diplosporid Coccidia only. As no *Eimeria* appeared in the faeces of this animal within a couple of days, an artificial infection was tried by giving it a large dose of sporulated oöcysts, collected from the faeces of badger No. I. Five days later the examination of the faeces revealed two oöcysts which in all morphological details agreed with those used for the infection prior to sporulation. On the seventh day after the administration of the infective material the faeces of the experimental animal contained large numbers of oöcysts belonging to *Eimeria* and in addition diplosporid Coccidia of the same type as that present prior to the artificial infection.

The course of this experiment is recorded in Table I.

It may be concluded from this experiment that the eimerid oöcysts previously found in two of the badgers which died at the Zoological Gardens belong to a species of *Eimeria* which may easily be transmitted to this host.

Attempts were also made to transmit this coccidium to cats. Young cats serving as experimental animals in the laboratory were repeatedly fed with sporulated oöcysts with negative results.

In view of the above experiments it seems reasonable to assume that the coccidium in question represents a species of *Eimeria* characteristic of the badger, for which we propose the name *Eimeria melis* n.sp. This species may be characterised as follows:

Eimeria melis n.sp.

The oöcysts are colourless, the cyst wall moderately thick; their shape is in general ovoid, less frequently somewhat rounded or elliptical. The size

varies between 17–24 × 13–17 μ, the majority are 19–21 μ long. Exceptionally oöcysts may be met with measuring 15–17 μ in length. The sporont in most cases occupies the greater part of the cyst, being but rarely of a definite rounded shape. No evident micropyle is present. Sporulation takes place, as a rule, within 2–4 days. The sporoblasts are mostly ovoid in shape, while the sporocysts containing developed sporozoites are more or less fusiform. In freshly sporulated oöcysts each sporocyst contains a small residual body, which, however, soon loses its definite contours. We have no data regarding the schizogonic and gametogonic development, or the location of the parasite within the intestinal tract. The prepatent period, judging from our experiments, seems to be 5 days.

Table I.

Date of administration of sporulated material: July 6.

Date of faecal examination	Oöcysts of	
	Eimeria	*Lucetina*
July 1	–	+
„ 5	–	+
„ 6	–	+
„ 11	+ (few)	+
„ 13	+ + +	+
„ 14	+ + + +	–
„ 15	+ + +	–
„ 18	+ + + +	–
„ 20	+ + +	–
„ 22	+ +	–
„ 25	+	+
„ 27	+	+
„ 31	+	+
Aug. 4	+ +	+
„ 10	+	+
„ 12	+	+
„ 19	+	+ +
„ 24	+	+

THE DIPLOSPORID COCCIDIUM (Figs. 5–8).

It has been previously stated that in three out of four badgers examined diplosporid Coccidia were found. Their oöcysts showed morphological features in general closely resembling those of *Lucetina rivolta* of cats and dogs. Nevertheless certain minor differences could be observed which, if not supported by biological characteristics, would scarcely be sufficient to justify any specific distinction between the diplosporid coccidium of the badger and the named species of cats and dogs. It should be borne in mind, however, that our knowledge on the latter species is very unsatisfactory. There is as yet no clear experimental evidence as to the specificity of the feline and canine *Lucetina rivolta*. In consequence it is difficult to form a definite opinion as to the position of this diplosporid species of the badger within the group of diplosporids of carnivorous animals.

With the object of obtaining experimental data on this subject we attempted (*a*) to infect a badger kept in our laboratory with diplosporid

Coccidia of cats, and (*b*) to infect cats with diplosporid material collected from the badger.

The results of these cross infections, based on serial faecal examinations, follow.

(1) *Attempts to infect the badger with feline Coccidia.*

As already mentioned the badger, brought to the laboratory for experimental purposes, was found to be moderately infected with a diplosporid coccidium resembling *Lucetina rivolta*. The course of the infection, from July 1st until nearly the end of August, as determined by faecal examinations, is shown in Table I (*ante*, p. 104). On September 7th, no diplosporid oöcysts having been detected for several days, a large quantity of sporulated material containing both *Lucetina felis* and *L. rivolta* was administered orally to the animal. Subsequent examinations gave the results recorded in Table II.

Table II.

Date of artificial infection	Date of faecal examination	Oöcysts of *Eimeria*	Oöcysts of *L. felis* type	Oöcysts of *L. rivolta* type
	Aug. 24	+	–	+
	Sept. 1	+	–	–
Sept. 7: with *L. felis* + *L. rivolta*	„ 7	+	–	–
	„ 12	+	–	–
	„ 14	+ +	–	–
	„ 15	+ +	–	–
Sept. 16: ditto.	„ 16	–	–	–
	„ 19	–	–	–
	„ 21	+	–	–
	„ 22	+	–	–
	„ 23	–	–	–
	„ 24	–	–	–
	Oct. 1	+ + +	–	+
	„ 12	–	–	–
Oct. 13: with diplosporid of the badger	„ 13	–	–	–
	„ 18	–	–	+
	„ 20	–	–	+
	„ 25	+ +	–	+
	„ 27	+	–	+
	„ 29	+	–	+

From the data in Table II there seems to be no doubt that our attempts to transmit diplosporid Coccidia of the cat to the badger failed, at least so far as *Lucetina felis* is concerned. The results with *L. rivolta* are not so clear, as the animal itself originally harboured Coccidia resembling this species; also it was not possible to assume that the natural infection had definitely disappeared at the time of the artificial infection. The fact, however, that following the administration of the sporulated material no diplosporid oöcysts of any type could be detected during 17 days, *i.e.* during a period sufficiently long for the development of gamogonic stages and for the appearance of oöcysts, tends to show that the second diplosporid species of the cat, *Lucetina rivolta*, is similarly not transmissible to the badger.

Nevertheless there are certain points which require explanation. Above

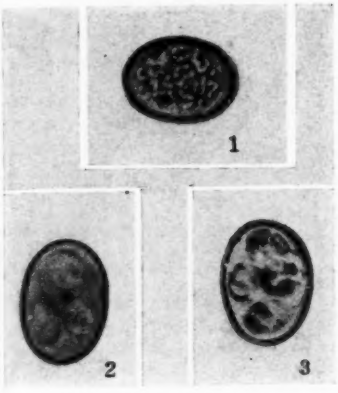
all the objection might be made that because of the omission of daily faecal examinations the data are not sufficiently complete to allow any conclusions to be drawn from them. It seems beyond doubt that as regards the course of the artificial infection the omission of daily examinations has no bearing upon the results obtained, for the appearance of oöcysts following the artificial infection could not be reasonably expected within 4–5 days. The above objection, however, might be admissible, if the faecal examinations were to determine the exact course of the original (natural) infection with diplosporids. It must be admitted that possibly more definite conclusions could have been drawn from the experiment, if full data concerning the latter conditions had been collected. According to the result of the faecal examination of October 1st, *i.e.* after more than 30 days since the last detection of diplosporid oöcysts, the appearance of a moderate number of oöcysts was noted. It seems hardly admissible to ascribe their appearance to the artificial infection. They might have been derived from an auto-reinfection to which the animal was subjected in spite of our efforts to keep its cage clean. This opinion seems to be in harmony with the observation that the oöcysts in question showed certain morphological details which were found to be more or less characteristic of the diplosporid form of the badger, as pointed out in the next chapter.

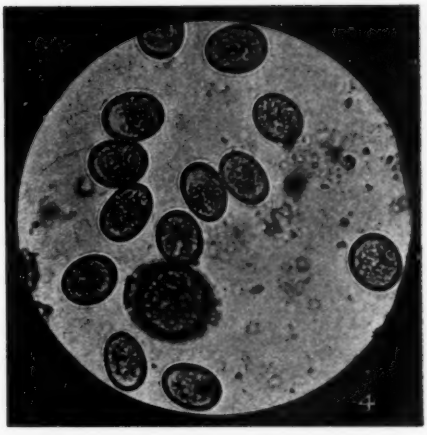
That the failure of a successful transmission of the feline diplosporids to the badger was not due to an acquired, post-infectional, immunity, seems to be evidenced by the fact that the oral administration of sporulated diplosporid material coming from the badger, was followed by the prompt, though by no means copious appearance of oöcysts of the same type.

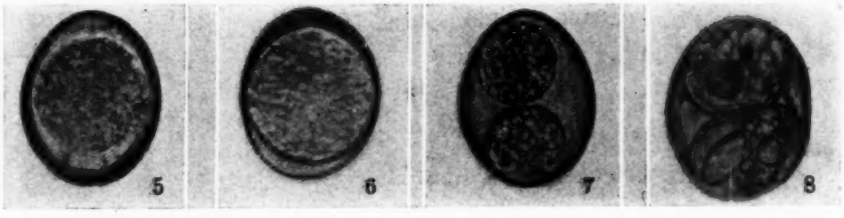
(2) *Attempts to infect cats with the diplosporid coccidium of the badger.*

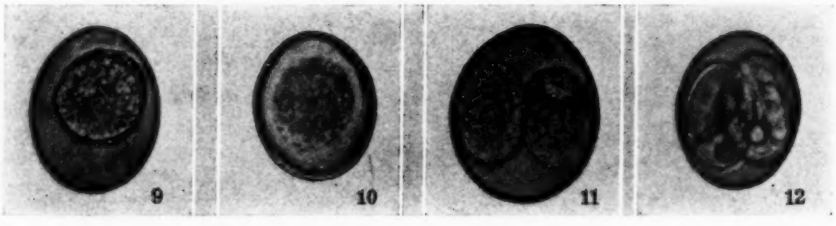
Although several attempts were made to transmit the diplosporid coccidium of the badger to cats, no reliable results could be expected owing to the circumstance that the young cats at hand almost invariably showed an infection with both *Lucetina felis* and *L. rivolta*. In addition we tried to utilise certain morphological differences between sporulating oöcysts of *L. rivolta* and the similar form of the badger. It was found that while the sporont of *Lucetina* sp. of the badger appears significantly larger than that of *L. rivolta*, in most cases it is the position of the sporoblasts within the oöcyst which may differ. The sporoblasts of *L. rivolta* of the cat and dog are usually (but not invariably) in a side by side position, while those of the badger diplosporid, in most cases, assume a so-called tandem position. On these differences it might be stated that our attempts to transmit the badger diplosporid to cats were not successful.

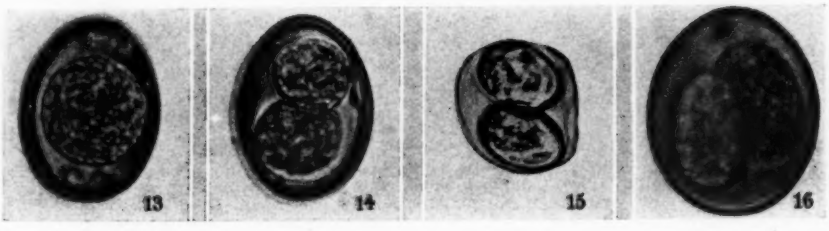
Owing to the lack of appropriate experimental material, it has, so far, not been possible to take into consideration *L. rivolta* of the dog. The identity of the latter with the closely related form of the cat being still uncertain, it will be necessary to examine separately the relations between the











UNIV. OF MICH.

badger diplosporid and *L. rivolta* of the dog. According to the present state of our knowledge the only statement that seems justified is that the diplosporid coccidium of *Meles taxus* is not identical with diplosporids occurring in cats.

EXPLANATION OF PLATE XII.

Figs. 1–3. Oöcysts of *Eimeria melis* n.sp. × 1000.
Fig. 4. Oöcysts of *Eimeria melis* n.sp. together with a single oöcyst of *Lucetina* sp. from the badger. × 500.
Figs. 5–8. Oöcysts of *Lucetina* sp. from the badger. × 1000.
Figs. 9–12. Oöcysts of *Lucetina rivolta* from the cat. × 1000.
Figs. 13–15. Oöcysts of *Lucetina canivelocis* from the silver fox. × 1000.
Fig. 16. Oöcyst of *Lucetina* sp. (*canivelocis*?) from the red fox. × 1000.

(*MS. received for publication* 11. XI. 1932.—Ed.)

NOTE ON THE OCCURRENCE OF *GRAFFILLA GEMELIPARA* LINTON (TURBELLARIA) AT PLYMOUTH.

By W. HAROLD LEIGH-SHARPE, M.Sc. (Lond.).

(With 1 Figure in the Text.)

On 28. viii. 1932 seven specimens of *Graffilla gemelipara* Linton 1910 were found by Miriam Rothschild on the body inside the mantle cavity of a specimen of *Cardium edule* from Millbrook, Plymouth.

Though only taken previously in America and on a different host, *Modiolus plicatulus*, Linton's excellent figure leaves no doubt that these specimens belong to the same species.

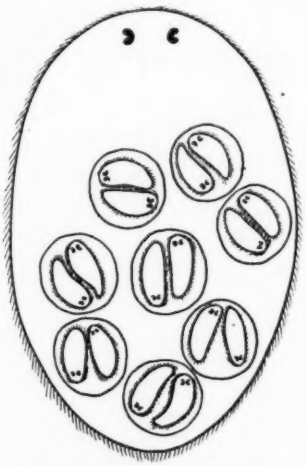


Fig. 1. *Graffilla gemelipara.*

The mother, a perfect oval, is uniformly ciliated, colourless and transparent, with a pair of typical Turbellarian bean-shaped eyes, and contains within her body eight bubble-like, transparent, spherical cysts. Each of these contains a pair of ciliated young, complete with eyes like their mother; and the young of each pair are arranged head to tail and slowly revolve within the cyst.

REFERENCE.

Linton, E. (1910). *J. Exper. Zool.* **9**, 371.

(*MS. received for publication* 18. x. 1932.—Ed.)

A LIST OF BRITISH FISHES WITH THEIR CHARACTERISTIC PARASITIC COPEPODA.

By W. HAROLD LEIGH-SHARPE, M.Sc. (Lond.).

The priority of the names of the fishes in the following list has been determined from Jordan in Part II of the *Report of the United States Commissioner of Fisheries for the year* 1928*: A check list of Fishes.* In determining the names of those not mentioned in this list I have to thank Mr J. R. Norman of the British Museum for his kind assistance.

Host	Author	Parasite	Author	Location
		(α) ELASMOBRANCHII.		
Alopias vulpinus	(Bonnaterre)	*Dinemoura producta*	(Müller 1785)	Surface of body
Carcharias glaucus	(Linn.)	*Echthrogaleus coleoptratus*	(Guérin 1829–43)	Surface of body
		Laminifera cornuta	(Milne-Edwards 1840)	Surface of body
		Pandarus bicolor	Leach 1816	Surface of body
Cetorhinus maximus	(Gunnerus)	*Nemesis vermi*	A. Scott 1929	Gills
		Dinemoura producta	(Müller 1785)	Skin
Cynias [*Mustelus*] *canis*	(Mitchill)	*Lernaeopoda mustelicola*	Leigh-Sharpe 1919	Cloaca
		Pandarus bicolor	Leach 1816	Surface of body
		Tripaphylum musteli	(van Beneden 1851)	Gills
Dasyatis [*Trygon*] *pastinaca*	(Linn.)	*Eudactylina minuta*	T. Scott 1904	Gills
		Charopinus bicaudatus	(Krøyer 1837)	Nasal fossae
Galeorhinus galeus	(Linn.)	*Lernaeopoda bidiscalis*	Kane 1892	Claspers
		L. galei	Krøyer 1837	Cloaca, claspers
		Pandarus bicolor	Leach 1816	Surface of body
		Kroyeria lineata	van Beneden 1853	Gill-rays
		Eudactylina insolens	T. and A. Scott 1913	Gills
Lamna nasus (=*cornubica*)	(Bonnaterre)	*Anthosoma crassum*	(Abilgaard 1794)	Upper jaw, surface
		Ommatokoita elongata	(Grant 1827)	Eye
		Dinemoura producta	(Müller 1785)	Surface of body
		Echthrogaleus coleoptratus	(Guérin 1829–43)	Surface of body
Raia batis	Linn.	*Caligus rapax*	Milne-Edwards 1840	Ventral surface
		Trebius caudatus	Krøyer 1838	Dorsal surface
		Acanthochondrites annulatus	(Olsson 1868)	Gills, nasal fossae
		Lernaeopodina similis	(T. and A. Scott 1913)	Gills
		Charopinus dalmanni	(Retzius 1829)	Nasal fossae
		C. dubius	T. Scott 1900	Pharynx
R. circularis	Couch	*C. dubius*	T. Scott 1900	Gills
R. clavata	Linn.	*Caligus rapax*	Milne-Edwards 1840	Ventral surface
		Charopinus ramosus	Krøyer 1863	Gills
R. fullonica	Linn.	*Taeniacanthus wilsoni*	A. Scott 1929	Gills
		Eudactylina similis	T. Scott 1902	Gills
		Lernaeopodina cluthae	(T. Scott 1900)	Gills
		Charopinus dubius	T. Scott 1900	Gills
R. maculata	Montague	*C. dubius*	T. Scott 1900	Gills
		C. ramosus	Krøyer 1863	Gills
R. oxyrhyncus	(Linn.)	*Thomsonella parkeri*	(Thomson 1889)	Gills
R. radiata	Donovan	*Eudactylina similis*	T. Scott 1902	Gills
		Lernaeopodina cluthae	(T. Scott 1900)	Gills
Scylliorhinus canicula	(Linn.)	*Lernaeopoda globosa*	Leigh-Sharpe 1918	Nasal fossae
		L. scyllicola	Leigh-Sharpe 1915	Cloaca, claspers
S. catulus	(Cuvier)	*Pandarus bicolor*	Leach 1816	Surface of body

Host	Author	Parasite	Author	Location
Squalus acanthias	Linn.	*Echthrogaleus coleoptratus*	(Guérin 1829–43)	Skin
		Eudactylina acanthii	A. Scott 1901	Gills
		Nogaus ambiguus	T. Scott 1907	Surface
		Demoleus paradoxus	(Otto 1828)	Surface
Squatina squatina	(Linn.)	*Eudactylina acuta*	van Beneden 1853	Gills
		(β) TELEOSTOMI.		
Acipenser sturio	Linn.	*Dichelesthium oblongum*	(Abilgaard 1794)	Gills
		Lepeophtheirus sturionis	(Krøyer 1837)	Surface of body
Agonus cataphractus	Linn.	*Haemobaphes cyclopterina*	(Fabr. 1780)	Gills
Anarrhichas lupus	Linn.	*Sphyrion lumpi*	(Krøyer 1845)	Surface
		Clavellodes rugosa	(Krøyer 1837)	Gills and operculum
Belone acus	Risso	*Bomolachus bellones*	Burmeister 1833	Gills
Callionymus lyra	Linn.	*Lernaeocera branchialis*	(Linn. 1767)	Gills, buccal cavity
		Clavellopsis quadrata	(Bassett-Smith 1896)	Gill-rakers
C. maculatus	Linn.	*Haemobaphoides ambiguus*	(T. Scott 1900)	Gills
		Disphaerocephalus ornatus	(T. Scott 1900)	Gills
Cantharus lineatus	(Montague)	*Alella canthari*	(Heller 1865)	Gills
Ciliata [*Onos*] *mustela*	(Linn.)	*Bomolochus onosi*	T. Scott 1902	Inside operculum
Clupea alosa	Linn.	*Lernaeenicus encrasicola*	(Turton 1807)	Body
		Clavellisa emarginata	(Krøyer 1837)	Gill-rakers
C. finta	Cuvier	*C. emarginata*	(Krøyer 1837)	Gill-rakers
C. harengus	Linn.	*Lernaeenicus sprattae*	(Sowerby 1806)	Eye
C. sprattus	Linn.	*L. sprattae*	(Sowerby 1806)	Eye
Coelorhyncus coelorhynchus	(Risso)	*Rebelula edwardsi*	(Kölliker 1853)	Surface
Conger conger	(Linn.)	*Congericola pallida*	van Beneden 1854	Gills
Cottus bubalis	Euphrasén	*Haemobaphes cyclopterina*	(Fabr. 1780)	Gills
C. scorpius	Linn.	*H. cyclopterina*	(Fabr. 1780)	Gills
Crenilabrus melops	(Linn.)	*Hatschekia pygmaea*	T. Scott 1907	Gills
Ctenolabrus rupestris	(Linn.)	*H. cluthae*	(T. Scott 1902)	Gills
Cyclopterus lumpus	Linn.	*Bomolochus soleae*	Claus 1864	Nostrils
		Sphyrion lumpi	(Krøyer 1845)	Tail
		Lernaeocera lumpi	(T. Scott 1901)	Gills
Cyprinus carpio	Linn.	*Lernaea cyprinacea*	Linn. 1761	Surface
Enchelyopus [*Onos*] *cimbrius*	(Linn.)	*Bomolochus onosi*	T. Scott 1902	Inside operculum
Engraulis encrasicholus	(Linn.)	*Lernaeenicus encrasicola*	(Turton 1807)	Eye
Esox lucius	Linn.	*Lernaea esocina*	(Burmeister 1833)	Surface
Gadus luscus	Linn.	*Bomolochus soleae*	Claus 1864	Nostrils
		Lernaeocera lusci	(Bassett-Smith 1896)	Gills
		Clavella typica	Leigh-Sharpe 1933	Gills
		Lernaeocera branchialis	(Linn. 1767)	Gills
G. merlangus	Linn.	*Clavella devastatrix*	Leigh-Sharpe 1925	Gills, pharynx, operculum
		Haemobaphes cyclopterina	(Fabr. 1780)	Gills
		Caligus curtus	Müller 1785	Surface of body
G. minutus	Linn.	*C. curtus*	Müller 1785	Surface of body
G. morrhua	Linn.	*Bomolochus soleae*	Claus 1864	Nasal fossae
		Caligus curtus	Müller 1785	Mouth
		C. rapax	Milne-Edwards 1840	Scales
		Lernaeocera branchialis	(Linn. 1767)	Gills
		Clavella iadda	Leigh-Sharpe 1920	Skin, fins, tail, anus
		C. sciatherica	Leigh-Sharpe 1918	Buccal cavity, pharynx
Gaidropsarus [*Onos*] *tricirratus*	(Bloch)	*Pseudocaligus brevipedes*	(Bassett-Smith 1896)	Inside operculum
Gastrosteus aculeatus	Linn.	*Thersetina gastrostei*	(Pagenstecher 1861)	Inside operculum
Germo alatunga	(Gmelin)	*Pennella germonia*	Leigh-Sharpe 1931	Surface
Gobius minutus	Gmelin	*Caligus rapax*	Milne-Edwards 1840	Scales
		Lernaeocera minuta	(T. Scott 1900)	Gills
Hippoglossoides platessoides	(Fabr.)	*Acanthochondria flurae*	(Krøyer 1863)	Gills

Host	Author	Parasite	Author	Location
Hippoglossus hippoglossus	(Linn.)	*Lepeophtheirus hippoglossi*	(Krøyer 1838)	Surface of body
		Hatschekia hippoglossi	(Krøyer 1838)	Gills
		Parabrachiella rostrata	(Krøyer 1837)	Gills
Labrax lupus	Cuv. and Val.	*Caligus minimus*	Otto 1828	Mouth and pharynx
		Lernanthropus kroyeri	van Beneden 1851	Gills
		Lernaeocera branchialis	(Linn. 1767)	Gills
Labrus berggylta (=*maculatus* Bloch)	Ascan.	*Caligus centrodonti*	Baird 1850	Tail and fins
		C. labracis	T. Scott 1902	Gills
		Hatschekia labracis	(van Beneden 1870)	Gills
L. mixtus	Linn.	*Caligus labracis*	T. Scott 1902	Gills
		Hatschekia labracis	(van Beneden 1870)	Gills
Lampris regius	(Bonnaterre)	*Thysanote lampri*	(T. and A. Scott 1901)	Gills
Lepidorhombus whiff	(Walbaum)	*Acanthochondria cornuta*	(Müller 1777)	Inside operculum
Limanda limanda	(Linn.)	*Lepeophtheirus pectoralis*	(Müller 1777)	Pectoral fins
		Acanthochondria limandae	(Krøyer 1863)	Gills
Lophius piscatorius	Linn.	*Chondracanthus lophii*	Johnston 1836	Opercular angle
Luvarus imperialis	Rafinesque	*Lutkenia asterodermi*	Claus 1864	Surface
Malacocephalus laevis	(Lowe)	*Chondracanthus neali*	Leigh-Sharpe 1930	Gills
Melanogrammus aeglefinus	(Linn.)	*Epibrachiella impudica*	(Nordmann 1832)	Gills
		Lernaeocera branchialis	(Linn. 1767)	Gills
		L. megalocephala	A. Scott 1929	Gills
		Clavella dubia	T. and A. Scott 1900	Gills
		C. brevicollis	(Milne-Edwards 1840)	Fins, body
Merluccius merluccius	(Linn.)	*Chondracanthus merluccii*	(Holten 1802)	Mouth, operculum
		Clavella deliciosa	Leigh-Sharpe 1933	Gills
		C. stellata	(Krøyer 1837)	Scales
		Parabrachiella insidiosa	(Heller 1865)	Gill-rays
		Brachiella merluccii	Bassett-Smith 1896	Gill-rakers
Microstomus kitt	(Walbaum)	*Acanthochondria clavata*	(Bassett-Smith 1896)	Gills
Mola mola	(Linn.)	*Cecrops latreillii*	Leach 1816	Gills
		Orthagoriscicola muricata	(Krøyer 1837)	Surface of body
		Philorthagoriscus serratus	(Krøyer 1863)	Surface of body
		Pennella orthagorisci	Wright 1870	Body
		Lepeophtheirus nordmanni	(M. Edwards 1840)	Surface
Molva molva	(Linn.)	*Bomolochus soleae*	Claus 1864	Nostrils
		Lepeophtheirus pollachius	Bassett-Smith 1896	Tongue, palate
Mugil chelo	Cuvier	*Ergasilus nanus*	van Beneden 1870	Gills
		Caligus rapax	Milne-Edwards 1840	Scales
Mullus barbatus	Linn.	*Hatschekia mulli*	(van Beneden 1851)	Gills
Pagellus centrodontus	(de la Roche)	*Alella canthari*	(Heller 1865)	Gills
		Caligus centrodonti	Baird 1850	Tail and fins
		Hatschekia cornigera	T. Scott 1909	Gills
Perca fluviatilis	Linn.	*Achtheres percarum*	Nordmann 1832	Gills
Pholis gunnellus	(Linn.)	*Lernaeocera branchialis*	(Linn. 1767)	Gills
		Haemobaphes cyclopterina	(Fabr. 1780)	Gills
Pleuronectes flesus	Linn.	*Lepeophtheirus pectoralis*	(Müller 1777)	Pectoral fins
		Lernaeocera branchialis	(Linn. 1767)	Gills
		Acanthochondria depressa	(T. Scott 1905)	Gills
		Bomolochus soleae	Claus 1864	Nostrils
P. platessa	Linn.	*B. soleae*	Claus 1864	Nostrils
		Lepeophtheirus pectoralis	(Müller 1777)	Pectoral fins
		Acanthochondria cornuta	(Müller 1777)	Inside operculum
Pollachius pollachius	(Linn.)	*Bomolochus soleae*	Claus 1864	Nostrils
		Clavella invicta	Leigh-Sharpe 1925	Gills
		Lepeophthirus pollachius	Bassett-Smith 1896	Buccal cavity
Salmo fario	Linn.	*Achtheres percarum*	Nordmann 1832	Gills
S. salar	Linn.	*Salmincola salmonea*	(Gissler 1751)	Gills
		Lepeophtheirus salmonis	(Krøyer 1837)	Surface of body
S. trutta	Linn.	*Caligus rapax*	Milne-Edwards 1840	Scales
Scomber scombrus	Linn.	*C. pelamydis*	Krøyer 1863	Inside operculum
		C. productus	Dana 1852	Inside operculum
		Clavellisa scombri	(Kurz 1877)	Gill-arches
		Clavellopsis paradoxa	(van Beneden 1851)	Gills
Scophthalmus maximus	(Linn.)	*Lepeophtheirus thompsoni*	Baird 1850	Gills

Host	Author	Parasite	Author	Location
Scophthalmus rhombus	(Linn.)	*Lepeophtheirus thompsoni*	Baird 1850	Gills
		L. hippoglossi	(Krøyer 1838)	Surface
Sebastes marinus	(Linn.)	*Haemobaphes cyclopterina*	(Fabr. 1780)	Gills
		Chondracanthopsis nodosus	(Müller 1777)	Opercular angle
Solea vulgaris	Quensel	*Bomolochus soleae*	Claus 1864	Dorsal surface
		Acanthochondria soleae	(Krøyer 1838)	Gills
Thunnus thynnus	(Linn.)	*Elytrophora brachyptera*	Gerstaecker 1853	Gills
		Brachiella thynni	Cuvier 1817	Behind pectoral fins
		Pennella sp.	—	Body
Trigla cuculus	Linn.	*Caligus diaphanus*	Nordmann 1832	Inside operculum
		C. gurnardi	Krøyer 1863	Pharynx
		Brachiella bispinosa	Nordmann 1832	Gill-rakers
		B. triglae	Claus 1860	Gills
		B. obesa	(Krøyer 1837)	Tips of gill filaments
		Epibrachiella impudica	(Nordmann 1832)	Inside operculum
		Oralien triglae	(Blainville 1822)	Gills
T. gurnardus	Linn.	*Caligus brevicaudatus*	A. Scott 1901	Gills, mouth
		C. gurnardi	Krøyer 1863	Gills
		Brachiella bispinosa	Nordmann 1832	Gill-rakers
		B. obesa	(Krøyer 1837)	Gill-arches
		B. triglae	Claus 1860	Gills
		Epibrachiella impudica	(Nordmann 1832)	Inside operculum
		Lernentoma asellina	(Linn. 1761)	Gills
T. hirundo	Bloch	*Caligus diaphanus*	Nordmann 1832	Inside operculum
		Brachiella triglae	Claus 1860	Gills
		Epibrachiella impudica	(Nordmann 1832)	Inside operculum
		Oralien triglae	(Blainville 1822)	Gills
T. lineata	Linn.	*Brachiella triglae*	Claus 1860	Gills
T. lyra	Linn.	*B. bispinosa*	Nordmann 1832	Gill-rakers
		Oralien triglae	(Blainville 1822)	Gills
T. obscura (=*lucerna*)	Linn.	*Caligus brevicaudatus*	A. Scott 1901	Inside operculum
Umbrina cirrosa	(Linn.)	*Scienophilus tenuis*	van Beneden 1852	Gill-chamber
Urophycis blennoides	(Brünnich)	*Lernaeocera phycidis*	Leigh-Sharpe 1933	Gills
		Alella alata	(Brian 1906)	Gill-arches
Xiphias gladius	Linn.	*Philichthys xiphiae*	Steenstrup 1862	Mucous canals of head
		Pennella sp.	—	Body
Zeugopterus punctatus	(Bloch)	*Bomolochus zeugopteri*	T. Scott 1902	Dorsal surface
Zeus faber	Linn.	*Caligus zei*	Norman and Scott 1906	Surface of body
		Chondracanthus zei	de la Roche 1811	Inside operculum, gills

(*MS. received for publication* 11. x. 1932.—Ed.)

A SECOND LIST OF PARASITIC COPEPODA OF PLYMOUTH WITH A DESCRIPTION OF THREE NEW SPECIES.

By W. HAROLD LEIGH-SHARPE, M.Sc. (Lond.).

(With 5 Figures in the Text.)

The following constitutes a supplement to my previous list (Leigh-Sharpe, 1926), and consists of a few records previously omitted, and also an account of some Copepoda taken by me during a visit to the Laboratory of the Marine Biological Association, Plymouth, Aug.–Sept. 1932.

The names of collectors given in the list have been abbreviated as follows: Br. = Prof. Emile Brumpt; E. = Richard Elmhirst; G. = Geoffrey W. Smith; L. = W. H. Leigh-Sharpe; N. = A. M. Norman and T. Scott; O. = C. L. Oakley; Sp. = E. R. Speyer.

Corrigenda.

(1) Since the previous list appeared the following species of *Chondracanthus*, viz. *C. clavatus*, *C. cornutus*, *C. depressus* and *C. soleae*, have been removed to the genus *Acanthochondria* and the species *C. annulatus* to the genus *Acanthochondrites* (*vide* Oakley, 1930).

(2) *Parasitology*, **18**, 385, line 34, for *Medisicaste asellinum* (Linnaeus) read *Oralien triglae* (Blainville).

Parasite	Author	Collector	Location	Host	See Note
		LICHOMOLGIDAE.			
Lichomolgus agilis	(Leydig)	L.	Gills	*Archidoris britannica* (=*tuberculata*)	A
Paranthessius rostratus	(Canu)	L.	Testis	*Cardium edule*	B
		ASCOMYZONTIDAE.			
Nicothoe astaci	Audouin and Milne-Edwards	N. and G.	Gills	*Astacus homarus*	—
		ERGASILIDAE.			
Bomolochus bellones	Burmeister	L.	Gills	*Belone acus*	—
		CALIGIDAE.			
Caligus labracis	T. Scott	L.	Gills	*Labrus berggylta*	C
Pseudocaligus brevipedes	(Bassett-Smith)	L.	Gills	*Onos mustela*	C
"	"	L.	Chalimus stages on fins	"	—
Trebius caudatus	Krøyer	O.	Dorsal surface	*Raia maculata*	C
		DICHELESTHIIDAE.			
Eudactylina acuta	van Beneden	L.	Gills	*Squatina squatina*	D
Hatschekia pygmaea	T. Scott	L.	Gills	*Crenilabrus melops*	E

Parasite	Author	Collector	Location	Host	See Note
		LERNAEIDAE.			
Lernaeocera branchialis	Linnaeus	L.	Gills	*Callionymus lyra*	F
L. phycidis	n.sp.	L.	Gills	*Urophycis blennoides*	—
		CHONDRACANTHIDAE.			
Acanthochondrites annulatus	(Olsson)	Sp.	Cloaca ♀	*Galeus vulgaris*	—
Lernentoma asellina	(Linnaeus)	L.	Gills	*Trigla gurnardus*	G
Oralien triglae	(Blainville)	L. and O.	Gills	*T. lyra*	C, G
		LERNAEOPODIDAE.			
Brachiella obesa	(Krøyer)	L. and O.	Tips of gill-filaments	*T. cuculus*	C
Clavella deliciosa	n.sp.	L.	Gills	*Merluccius merluccius*	—
C. typica	n.sp.	L.	Gills	*Gadus luscus*	H
Charopinus dalmanni	(Retzius)	L.	Nostrils	*Raia batis*	—
C. dubius	T. Scott	L.	Gills	*R. maculata*	—
,,	,,	L.	Pharynx	*R. batis*	—
C. ramosus	Krøyer	L.	Gills	*R. clavata*	I
		SPLANCHNOTROPHIDAE.			
Splanchnotrophus gracilis	Norman and Hancock	E.	Skin of body	*Acanthodoris pilosa*	—
		SACCOPSIDAE.			
Saccopsis alleni	Brumpt	Br.	—	*Polycirrus caliendrum*	—

Notes on species in the list.

A. Innumerable on all the eight *Archidoris* examined.

B. Innumerable on all the eight *Cardium* examined from Millbrook. Previously seen in 1917 and 1918.

C. New host in addition to previous record.

D. Fifteen specimens on one host from Rame-Eddystone.

E. About a hundred specimens on one host from Wembury.

F. A catch of about a hundred *C. lyra* from the Rame-Eddystone grounds. Out of fifty females examined none bore parasites; the only two males in the catch bore seven and eight respectively (4 + 3 and 4 + 4). New situation on same host.

G. Two on four *T. gurnardus*[1], two on eight *T. lyra*. Nothing was found on about a hundred *T. cuculus* examined.

H. Two on about ten specimens examined.

I. *Vide Parasitology*, **22**, 144.

Additional notes on species previously recorded.

(1) *Congericola pallida* occurred on the gills of two out of ten conger eels examined and on one individual twenty specimens were found. It is doubtful, therefore, whether it should be described as rare.

[1] Since the validity of *Lernentoma asellina* has been established (*Parasitology*, **19**, 455) it has been recorded on no species of *Trigla* other than *T. gurnardus*. It is extremely doubtful if Bassett-Smith found it on any other species. He was probably confusing it with *Oralien triglae*.

(2) On fifty flat-fish belonging to seven species no parasites were found except single females of *Acanthochondria clavata* on each of five *Pleuronectes microcephalus*, and which accordingly seems to be common.

(3) Monogenetic Trematodes were found in greater abundance on the gills of fishes than parasitic Copepoda and seemed mutually exclusive: *e.g.* on six *Gadus merlangus* taken haphazard from one catch, four bore *Octobothrium merlangi* and the other two *Clavella devastatrix*.

(4) Hitherto no parasitic Copepod has been recorded from *Cepola rubescens*, of which I examined twelve specimens, nor on *Trachurus trachurus* of which I had two specimens. I was not able to find any Copepods but I obtained Trematodes (which E. I. Jones will describe in the next number of this Journal).

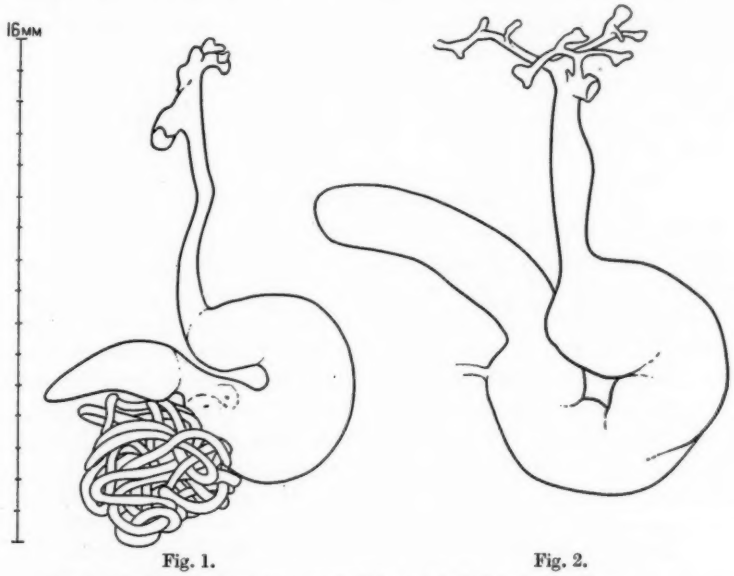


Fig. 1. Fig. 2.

Fig. 1. *Lernaeocera lusci* ♀, an unusual but probably normal specimen.
Fig. 2. *Lernaeocera phycidis* ♀, n.sp.

(5) Members of both sexes of *Caligus rapax* were constantly found free in the plankton.

(6) *Notodelphys allmani*, one female, commensal in the atrium of the only *Ascidiella aspersa* examined. Eggs a bright grass-green which changed to a vivid scarlet on immersion in alcohol.

NOTE ON *LERNAEOCERA LUSCI* (BASSETT-SMITH).

I have previously remarked upon and particularly discussed (*vide* Leigh-Sharpe, 1925) the fact that the antlers and horns of the Lernaeidae cannot be regarded as important diagnostic characters owing to the variability of their

configuration and degree of bifurcation. When one or more antlers come into contact with a bone a peculiar malformation results which is so far characteristic in *Lernaeocera* that it takes the form of one antler elongating enormously, running parallel to the bone and becoming very unlike the others. I am convinced that T. and A. Scott (1913) drew their figure of *L. lusci* (Pl. XLIV, fig. 1) from such a malformed specimen, and in fact I have found several specimens like it. However, on 29. viii. 1932, on the gills of *Gadus luscus* at Plymouth, I discovered a specimen of *Lernaeocera* whose antlers had not penetrated to the bone, and found myself confronted by an animal (Fig. 1) which had the antlers of *L. minuta*, embedded only in the gill filaments, and the body of *L. lusci*. Not wishing to cast doubt upon the validity of the species *L. minuta*, which I have not been able to find on *Gobius*, I have assigned this specimen to *L. lusci*, regarding the body form as more characteristic for diagnosis than the antlers. I have also been guided by the host.

To make matters more complicated I have found a *Lernaeocera* with the antlers of *L. branchialis* and the body form of *L. lusci*, which I am assigning to a new species as follows:

Lernaeocera phycidis n.sp.

Habitat and **Material.** One female specimen on the gills of *Urophycis blennoides*, one of nearly a hundred specimens examined from Milford Haven, 6. viii. 1932.

Diagnosis (Fig. 2). Antlers of the cephalon three and dichotomously branched, resembling *L. branchialis*. Neck without annulations, 9 mm. in length. Trunk red, swollen, at first abruptly reflexed and bent upon itself except at the distal end, where it is only slightly curved as in *L. lusci*. The terminal portion is more dilated than in *L. lusci*, and marked off from the trunk by a slight constriction. Ovisacs wanting.

Host. *Urophycis blennoides*.

Notes on the eggs and Nauplii of the Dichelesthiidae.

All the above-mentioned three members of the Dichelesthiidae immediately parted with their egg-strings post-mortem on immersion in alcohol. No males were observed. Each lived with the others of its species in sea water in a small watch-glass for 36 hours after removal from its host.

In *Hatschekia pygmaea* the number of uniserial eggs in each string varied from six to fifteen, the minimum and maximum numbers being the most frequent. One anomalous specimen had four eggs in one string and fifteen in the other. One *Congericola pallida* had nauplii escaping from its egg-strings. I do not think nauplii have been previously observed in any British Dichelesthiidae. Each nauplius was very active, much inflated (Fig. 3 A) shaped like a cottage loaf with its appendages very much to the anterior end. I was unable to observe any eye. After death and preservation the inflated cuticle collapsed (Fig. 3 B).

Genus **Clavella** Oken 1815.

(Following the system adopted in *Parasitology*, **17**, 194.)

C. deliciosa n.sp.

Host. *Merluccius merluccius*, gills not skin.

Locality. Plymouth.

Diagnosis. Cephalothorax, elongated, one and a third times as long as trunk, slender, straight and making a right angle with the trunk as in *C. stellata*[1], which is found on the same host but attached to a scale of the surface of the body by means of a characteristic discoid bulla, which distinguishes it from the present species with a balloon-like bulla embedded in the gill filaments. Channellings of bulla four on each side. Head not enlarged. Base of neck bulbous and very much enlarged. Trunk elongated, twice as

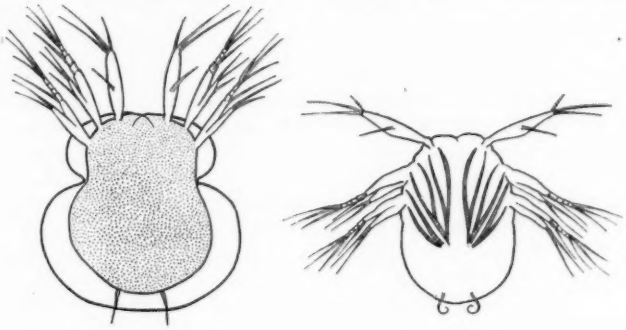


Fig. 3. *Congericola pallida*, nauplii. A, from a living specimen; B, from a Balsam-mounted slide.

long as broad. Second maxillae unusually long for this genus and situated at some distance from the anterior end of the trunk. Genital process robust. Ovisacs one and a third times as long as the trunk, tapering (Fig. 4).

Males unknown.

C. typica n.sp.

Host. *Gadus luscus*, gills.

Locality. Plymouth.

Diagnosis. A very small species. Cephalothorax elongated, slender, one and a half times as long as trunk and making a right angle with it. Head not enlarged. Base of neck not differentiated. Second maxillae very short but expanded into large disc. Bulla spherical but small in proportion to disc. Genital process elongate. Ovisacs unusually long, two and a half times as long as trunk (but this may be abnormal and there is only one specimen as yet taken) with rounded ends, not tapering (Fig. 5).

Males unknown.

[1] An error was made in quoting this point in *Parasitology*, **17**, 199.

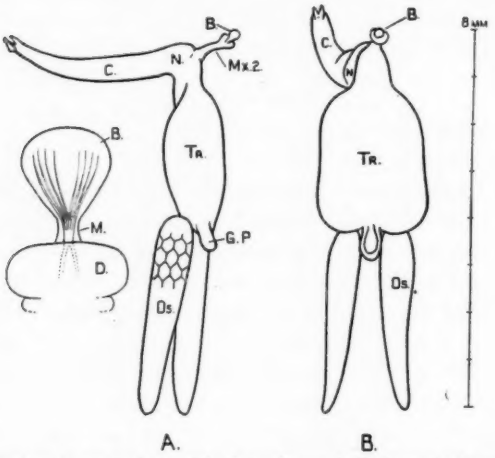


Fig. 4. *Clavella deliciosa* ♀, n.sp. A, in right lateral aspect; B, in ventral aspect; *C.* = cephalothorax; *N.* = neck; *Tr.* = trunk; G.P. = genital process; *Os.* = egg-strings; *B.* = bulla; *M.* = manubrium; *D.* = disc; *Mx.2.* = second maxillae.

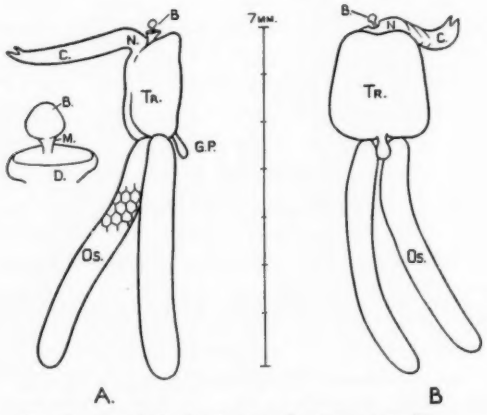


Fig. 5. *Clavella typica* ♀, n.sp. Lettering as in Fig. 4.

REFERENCES.

LEIGH-SHARPE, W. H. (1925). *Lernaea elegans. Parasitology*, **17**, 250.

—— (1926). A list of parasitic Copepoda occurring at Plymouth. *Ibid.* **18**, 384.

OAKLEY, C. L. (1930). The Chondracanthidae...with a description of five new genera and one new species. *Ibid.* **22**, 182.

SCOTT, T. and SCOTT, A. (1913). *British Parasitic Copepoda.* Ray Society, London.

(*MS. received for publication* 28. IX. 1932.—Ed.)

THEOBALDIA (*CULICELLA*) *LITOREA* (SHUTE) N.SP. (DIPTERA, CULICIDAE).

By J. F. MARSHALL, M.A., F.E.S. and J. STALEY, F.E.S.
British Mosquito Control Institute, Hayling Island.

(With 2 Figures in the Text.)

In *The Entomologist* of August, 1928, P. G. Shute gave an account of mosquitoes reared from larvae collected by him in salt marshes at Walton-on-the-Naze (Essex), which, he stated, though closely resembling *Theobaldia* (*Culicella*) *morsitans* Theo., differed from that species in certain respects, notably by having the male adult palps distinctly shorter. The description given by Shute of this "short-palped" *Culicella* was as follows:

Larvae. Dark brown in colour, pecten teeth more widely spaced than in typical *C. morsitans* and sometimes extending as far as one-third the length of the siphon. Comb scales taper sharply at the base. Anal gills shorter, hardly more than one-third the length of the anal segment.

Adult ♀. Proboscis uniformly speckled with creamy white scales at the side and beneath. Legs with tarsal joints banded, closely resembling *C. fumipennis*. Scutellum brown, clothed with whitish yellow scales. Abdomen dark brown, with basal yellow bands. Cerci yellow.

Adult ♂. Palpi only slightly longer than the proboscis, the last two segments less hairy than in typical *C. morsitans*. Proboscis yellow except at the tip.

Hypopygium as in typical *C. morsitans*; side pieces about three times as long as their basal width, the tip of the eighth sternite with an irregular row of stout bristles.

It was decided to regard this short-palped *Culicella* as a variety of *Theobaldia* (*Culicella*) *morsitans*; and, since the material described had originated in a coastal marsh, the varietal name *litorea* was chosen. The mosquito therefore entered the British list as *Theobaldia* (*Culicella*) *morsitans* var. *litorea* Shute.

During the past four years, *Culicella* larvae collected at Sandwich (Kent) and Hayling Island (Hampshire) have provided us with much material for examination. In the course of our investigations, *Culicella* larvae (other than those of *C. fumipennis*) were separately reared; each male adult produced, whether long-palped (*morsitans*) or short-palped (*litorea*), being preserved in the company of the corresponding larval skins.

When the palps are viewed alongside the proboscis, the difference of length in the two cases is very easily seen. The last joint of the palp (*i.e.* the junction of its last two segments) falls conspicuously short of the extremity of the labium in *litorea*, and overlaps it equally obviously in both *morsitans* and *fumipennis*. Of the specimens examined by us, we find that, if the length of the labium be taken as 100, the combined length of the first four segments

of the palp varies from 102·5 to 106 in *fumipennis*, from 101 to 110 in *morsitans* and from 89 to 96·5 in *litorea*. The ratio obtained by dividing the length of the last segment of the palp by the total length of the palp has an average value of 0·43, 0·44 and 0·46 in *fumipennis*, *morsitans* and *litorea* respectively. The relative measurements of some of the specimens examined are compared diagrammatically in Fig. 1.

The vestiture of the palps is also noticeably different in the three cases, the hairs on the third and fourth segments of *litorea* being distinctly fewer and stouter than those of *fumipennis*, while those of *morsitans* are of an intermediate character.

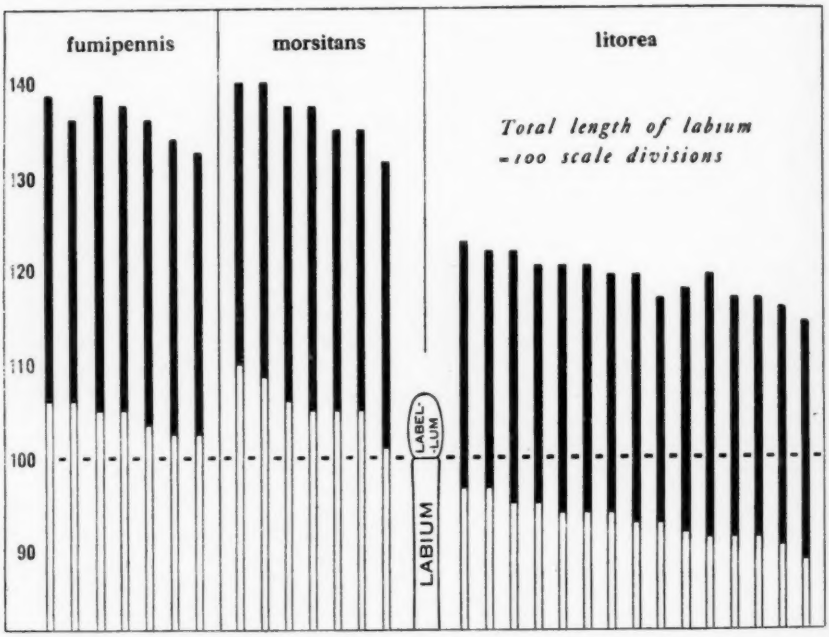


Fig. 1. Relative variations in length of the male palp, and of its last segment (indicated in black) in the three British species of *Culicella*.

A careful comparison of the two collections of material thus prepared shows that the larval characteristics upon which Shute relied have little, if any, diagnostic value. Neither the colour of the larvae nor the spacing of their pecten teeth enables the separation of *morsitans* from *litorea* to be effected; while comparative measurements of the length of the pecten are scarcely more helpful. In the fourth instar, for instance, we find that, if the length of the siphon be taken as 100, that of the pecten varies from 12 to 25 in *morsitans* and from 13 to 28 in *litorea*—the two ranges of variation being thus almost identical.

Diagnosis based upon the shape of the comb scales is equally unsatisfactory, since basally tapering and basally blunt forms occur together both in the comb of *morsitans* and in that of *litorea*. As regards the length of the anal gills, this is of course much too variable a factor to be made use of for differentiation when the larvae (as in this case) have been taken from waters of different degrees of salinity.

The only satisfactory larval distinction that we have so far noted is furnished, in the later instars, by the length of the siphonal tuft. If, as before, the length of the siphon be taken as 100, the length of the tuft of *litorea* will be found to vary from 40 to 57, from 40 to 50 and from 40 to 48, in the second, third and fourth instars respectively. In the case of *morsitans*, the corresponding ranges of variation are 34 to 37, 34 to 44 and 32 to 42.

In the first instar, a satisfactory means of distinguishing *litorea* and *morsitans* from one another has yet to be found. It may, however, be mentioned that, in the case of nearly all the first instar larvae hitherto examined, both the sub-basal and the infra-orbital hairs are simple in *litorea* and bifid in *morsitans*.

In the adult form, as Shute points out, *litorea* bears a much closer resemblance to *fumipennis* than it does to *morsitans*; indeed, his description of the adult female would apply equally well to either the first—or the second—mentioned mosquito. We find, however, that if, in a mixed batch of female adults, the specimens of *morsitans* are identified first of all (by noting the absence of pale scales from the proboscis and of the "inverted V" pattern from the abdominal sternites), the specimens of *fumipennis* and *litorea* which remain may then be separated by comparison of hind-leg markings; *fumipennis* having conspicuous yellow rings at all tarsal joints, whereas at the last two tarsal joints of *litorea* these rings are less conspicuous if not entirely absent.

In the case of male adults, the separation of *fumipennis*, *morsitans* and *litorea* from one another is a comparatively easy matter in view of the evidence provided by (1) the ventral markings, (2) the vestiture and length of the palps and (3) the various hypopygial characteristics about to be described. A further important diagnostic indication is the ratio obtained by dividing the length of the first fore-tarsal segment into the total length of the remaining four segments[1]. The value of this ratio is 1·06 in *fumipennis*, 0·8 in *morsitans* and 0·9 in *litorea*.

The most remarkable part of Shute's description is, however, the statement that the hypopygium of *litorea* is "as in typical *C. morsitans*." As will be seen from Fig. 2, the hypopygium of *litorea* differs far more from that of either *fumipennis* or *morsitans* than the two last-mentioned differ from one another. Whereas, for instance, the ratio of the length of the coxite to its basal width is about 2·5 and 2·4 in the case of *fumipennis* and *morsitans* respectively, this ratio is only about 2·1 in the case of *litorea*. Again, both in *fumipennis* and *morsitans* three or four of the spines on the basal lobe are conspicuously stout,

[1] This method of distinguishing *fumipennis* from *morsitans* was pointed out by F. W. Edwards in 1912.

but none of these spines reaches anywhere near to the tip of the coxite. In *litorea*, on the other hand, the basal lobe carried two conspicuous spines, the longer of which (if suitably directed) extends as far as, if not well beyond, the tip of the coxite.

The proportions of the style differ markedly in the three cases. If the breadth of the style at its centre be divided by its length the ratio will be found to be about 0·06 in *fumipennis*, from 0·08 to 0·09 in *morsitans* and from 0·04 to 0·05 in *litorea*. The style of *morsitans* is thus conspicuously stouter than that of *fumipennis*—a fact which appears to provide the best (not to say the only) means of distinguishing the hypopygia of these two species from one another.

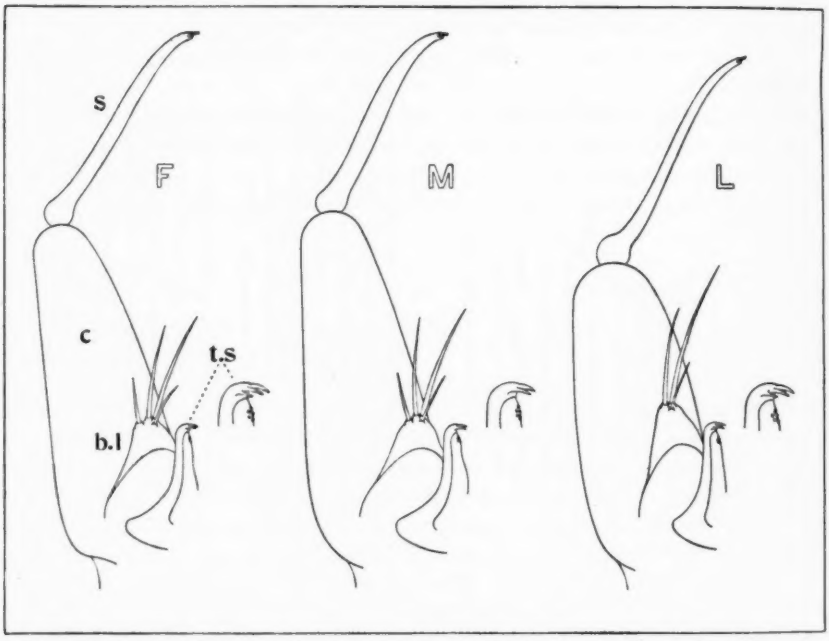


Fig. 2. Distinctive hypopygial characteristics of *Culicella fumipennis* (*F*), *morsitans* (*M*), and *litorea* (*L*). Magnification 112. *b.l*, basal lobe; *c*, coxite; *s*, style; *t.s*, tip of tenth sternite.

The ratio of the length of the style to that of the coxite was also determined in a large number of specimens, the results in the cases of *fumipennis* and *morsitans* being very similar; the range in the former being from 0·63 to 0·73 with an average of 0·68, and that in the latter being from 0·63 to 0·70 with an average of 0·65. In the case of *litorea*, however, the ratio varies between the limits of 0·80 and 0·88, with an average of 0·84.

A further diagnostic indication is possibly provided by the basal extremity of the style, which appears to be distinctly more bulbous in *litorea* than in either *fumipennis* or *morsitans*.

In addition to the above-mentioned hypopygial distinctions, which we consider to be invariable, there are others which are present in so large a majority of cases that they often afford valuable confirmation of evidence already considered. Notably is this so in the case of the teeth at the tip of the tenth sternite, the number of which is nearly always two in *morsitans* and nearly always three in *fumipennis* and *litorea*.

To facilitate comparison between the larval, adult and hypopygial distinctions above described, these have been summarised in parallel columns in Table I.

Dr F. W. Edwards, who has kindly examined some of the typical specimens referred to in this paper, agrees that the hypopygial characteristics of *litorea* are sufficient to justify its elevation to species rank. It will therefore be known as *Theobaldia* (*Culicella*) *litorea* (Shute).

A few notes regarding the ecology of this species may be of interest. As already stated, its larvae have been collected at Walton-on-the-Naze, Essex (1928, P. G. Shute), Sandwich, Kent (1930, J. Staley), and Hayling Island, Hampshire (1929–32, British Mosquito Control Institute). Some larvae have also been collected recently in the neighbourhood of Belfast (1932, Prof. Gregg Wilson). A description of the principal sources from which our material was obtained is given in Table II. The figures indicating salinity are percentages, the salinity of sea water of an assumed chlorine content of 19·315 parts per 1000 being taken as 100.

The fact that all larval material of *C. litorea* so far obtained has been collected exclusively in the near neighbourhood of the sea has, until recently, served to strengthen the original belief regarding the maritime character of this species. We have now, however, found, among specimens labelled "*C. morsitans*" in the Cambridge University Museum collection, two undoubted male adults of *C. litorea* taken by the Rev. L. Jenyns, at Swaffham Bulbeck, a village near Cambridge, nearly a century ago. In the same collection there is also another male of this species among specimens labelled "F. Jenkinson, Cambridge, 1920[1]." It appears probable, therefore, that further records of *C. litorea* from inland districts will follow the publication of the diagnostic characteristics hereinbefore described. As shown in Table II, the larvae of this species have been collected both in fresh water and in water of varying degrees of salinity up to 15/100 that of sea water. The same range of salinity is tolerated by the larvae of *C. morsitans*, a species which appears to breed equally freely in seaside or inland localities. It is perhaps important to note that, whereas the larvae of *C. morsitans* are commonly met with both in open and densely shaded situations, those of *C. litorea* have hitherto been found in pools of the former character only.

[1] We are greatly indebted to Mr G. J. Kerrich, of the Cambridge Zoological Museum, both for the loan of these specimens and for permission to make microscope preparations of their hypopygia.

Table I. *Chief points of difference between British species of the sub-genus* Culicella.

Larva	*C. fumipennis*	*C. morsitans*	*C. litorea*
Siphon:			
Ratio $\frac{\text{Length of tuft}}{\text{Length of siphon}}$			
Second instar	0·54–0·64*	0·34–0·37	0·40–0·57
Third instar	0·70–0·78*	0·34–0·44	0·40–0·30
Fourth instar	0·46–0·54*	0·32–0·42	0·40–0·48
Adult, female			
Proboscis:			
Coloration	Dark, middle third with pale scales laterally and below	Uniformly dark, or with very few pale scales on middle third	Middle third with pale scales as in *fumipennis*
Legs:			
Markings	Conspicuous yellowish rings at all tarsal joints	Rings at last two tarsal joints inconspicuous or absent in fore and mid-leg, and absent in hind leg	Rings at last two tarsal joints less conspicuous than in *fumipennis* on fore and mid-leg, and inconspicuous or absent in hind leg
Abdomen:			
Pattern on venter	"Inverted V" markings usually present	"Inverted V" markings rarely present	"Inverted V" markings not uncommon
Adult, male			
Maxillary palps:			
Character of vestiture	Third and fourth segments bearing numerous fine hairs	Hairs on third and fourth segments fewer and thicker than in *fumipennis*	Hairs on third and fourth segments fewer and thicker than in *morsitans*
Length to fourth joint (labium = 100)	102·5–106	101–110	89–96·5
Fore-legs:			
Ratio $\frac{\text{Length of last 4 tarsal segments}}{\text{Length of first 4 tarsal segments}}$	1·06	0·8	0·9
Abdomen:			
Pattern on venter	As in female	As in female	As in female

Table I (*continued*)

Male Hypopygium			
Coxite:			
Shape of tip	Tapering	Tapering	Blunt
Ratio $\frac{\text{Length of coxite}}{\text{Basal width of coxite}}$	2·4	2·5	2·1
Basal lobe:			
Number and character of spines	3 or 4 conspicuously stout, each falling a long way short of tip of coxite	As in *fumipennis*	2 conspicuously stout, one able to reach as far as, or beyond, tip of coxite
Ratio $\frac{\text{Length of lobe}}{\text{Length of coxite}}$	0·38	0·41	0·51
Style:			
Shape at basal end	Slightly bulbous	Slightly bulbous	Distinctly bulbous
Ratio $\frac{\text{Central breadth of style}}{\text{Length of style}}$	0·06	0·08–0·09	0·04–0·05
Ratio $\frac{\text{Length of style}}{\text{Length of coxite}}$	0·68	0·65	0·84
Tenth sternite:			
Number of teeth at tip	3 (rarely 4 or 5)	2 (rarely 3)	3 (rarely 2)

* In all larval instars, *C. fumipennis* is, of course, at once distinguished from both *C. morsitans* and *C. litorea* by the conspicuous isolated spines which form the distal part of its pecten.

Table II. *Principal habitats of the larval material from which adult specimens were reared for the purpose of this investigation.*

1. Hayling Is.	Pond near railway station, 150 yards from sea. Salinity 0. All males reared were *litorea*.
2. Hayling Is.	Collection of seepage water about 350 yards from sea. Salinity 0. Proportion of *litorea* males to *morsitans* males reared, 3 to 1.
3. Sandwich.	Water in dyke about ½ mile from sea. Salinity 0. Of 21 male adults reared, 19 were *litorea* and 2 *morsitans*.
4. Hayling Is.	Various pools about 100 yards from sea. Salinity variable. 55 males reared, 48 being *litorea* and 7 *morsitans*.
5. Hayling Is.	In a ditch adjoining sea wall. Salinity 0. Together with larvae of *Anopheles claviger*, *Aedes detritus*, *Aedes rusticus* and *Theobaldia annulata*. All males reared were *morsitans*.
6. Hayling Is.	Pool at edge of wood, near sea. Salinity 8. Together with larvae of *Aedes detritus*, *Aedes rusticus* and *Theobaldia annulata*. Males reared were both *litorea* and *morsitans*.
7. Witley (Surrey).	Woodland pool, well shaded. Together with larvae of *C. fumipennis*, *Aedes punctor* and *Aedes cinereus*. All males reared were *morsitans*.
8. Hayling Is.	Open woodland pool, 100 yards from sea. Salinity 0. Of 25 males reared, 13 were *litorea* and 12 *morsitans*.
9. Hayling Is.	Open pool near sea. Salinity 0. Together with larvae of *C. fumipennis*, *Aedes detritus*, *Aedes rusticus*. Of 32 males reared, 19 were *litorea* and 13 *morsitans*.
10. Hayling Is.	Open pool near sea. Salinity 5. Of 23 males reared, 20 were *litorea* and 3 *morsitans*.
11. Hayling Is.	Flooded margins of pond, under willow bushes. Males reared were *litorea* and *morsitans*.
12. Hayling Is.	Densely shaded moat in wood. Salinity 0. Together with larvae of *Aedes maculatus*. All males reared were *morsitans*.
13. Hayling Is.	Pool with rushes, among willow bushes. Salinity 15. Together with larvae of *Anopheles claviger*, *Aedes detritus*, *Theobaldia annulata*, *Theobaldia subochrea*. Of 23 males reared, 22 were *litorea* and 1 *morsitans*.
14. Hayling Is.	Open pool. Salinity 15. All males reared were *morsitans*.
15. Sutton Coldfield (Warwickshire).	Various pools, both open and in semi-shade. All males reared were *morsitans*.
16. Hayling Is.	Pool among bushes, near sea. Salinity 3. All males reared were *morsitans*.
17. Hayling Is.	Densely shaded woodland pool. Salinity 0. Together with larvae of *Aedes maculatus* and *Aedes rusticus*. All males reared were *morsitans*.

(*MS. received for publication* 14. IX. 32.—Ed.)

A NEW SPECIES OF *POLYPLAX* (ANOPLURA).

By G. F. FERRIS.

Stanford University, California.

(With 2 Figures in the Text.)

The new species herein described, collected by Mr Charles Elton in the course of ecological field work in Norway, was very kindly passed on by him to me for identification and description.

Polyplax borealis n.sp.

Material examined. Holotype, a female, and allotype and one paratype male from *Evotomys* sp. (*rufocanus*?), Beskenjarrga, Finmark, Norway, *C.*

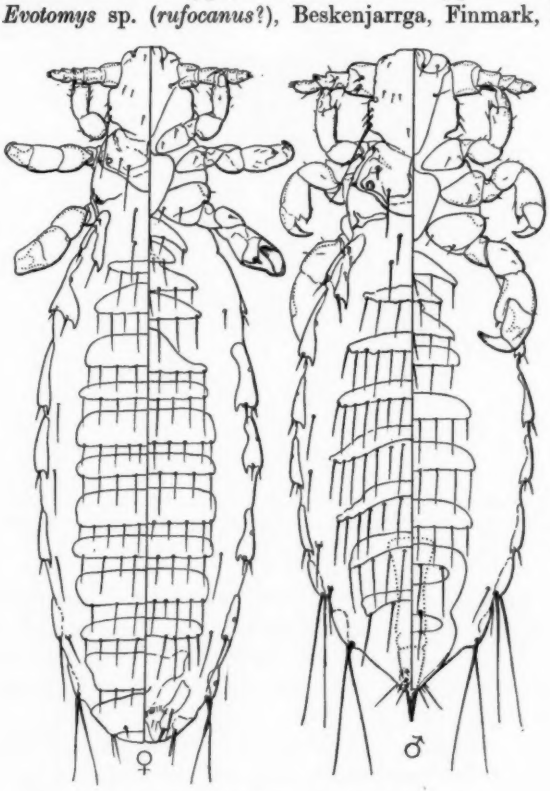


Fig. 1. *Polyplax borealis* n.sp. Female and male.

Elton. Type and holotype in the Oxford University collection, paratype in the Stanford University collection.

Female (Fig. 1). A species of the *P. spinulosa* group, distinguished from its near relatives by the following characteristics. *Sternal plate* (Fig. 2 *B*) longer than wide, its sides converging smoothly to a bluntly rounded apex. *Paratergal plates* (= pleurites) of the abdomen (Fig. 2 *A*) of the third to sixth segments each with the posterior lateral angles produced into a short, fimbriate point, those of the seventh and eighth segments without these points; setae

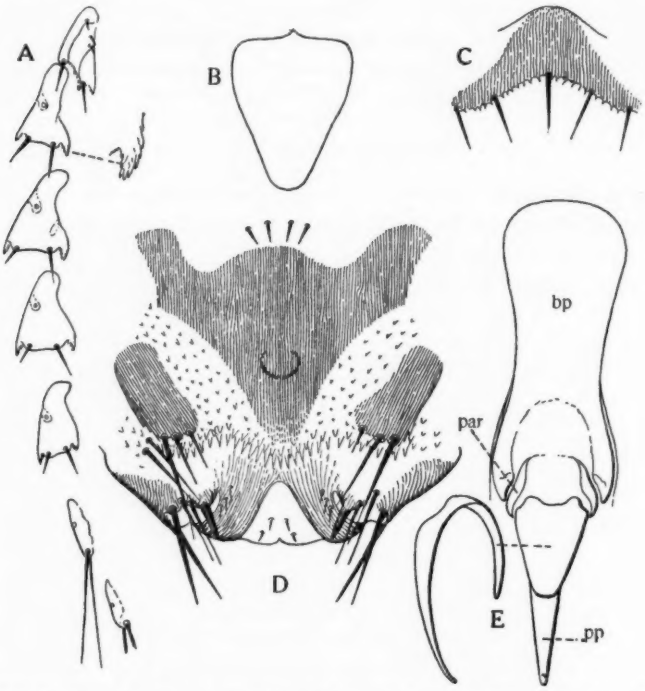


Fig. 2. *Polyplax borealis* n.sp. *A*, paratergal plates of female. *B*, sternal plate of thorax. *C*, first apparent sternite of abdomen of female. *D*, genital region of female. *E*, genitalia of male.

of all the plates, except seventh and eighth scarcely half as long as the plate, rather stout, the dorsal seta slightly the shorter. First sternal plate of the abdomen (Fig. 2 *C*) strongly arcuate. *Genital region* (Fig. 2 *D*) essentially as in *P. spinulosa* but with the gonopophyses narrower and the median plate somewhat differently formed.

Male (Fig. 1). Length 1 mm. Differing from the males of related species in the following details. *Genitalia* (Fig. 2 *E*) with the parameres (*par*) extremely small and the pseudopenis (*pp*) extraordinarily large, the latter viewed laterally

being in the form of a hook, its basal portion being bent upward and posteriorly and equalling nearly half the total length of the pseudopenis; basal plate (*bp*) quite broad, the apices of its arms partially enclosing the parameres. *Tergal plates* of the abdomen for the most part curved and with the posterior margin strongly arcuate, the first sternite with its posterior margin as in the female.

Note. It is not impossible that this species is *Polyplax alaskensis* Ewing, which was described from a single male from a related host in Alaska, but the description of this is entirely inadequate and is unaccompanied by figures. *P. borealis* is clearly distinct, on the basis of the characters which have been given, from any other member of the genus.

(*MS. received for publication* 4. XI. 1932.—Ed.)

THE HELMINTHS PARASITIC IN A WATER MOCCASIN (SNAKE) WITH A DISCUSSION OF THE CHARACTERS OF THE PROTEOCEPHALIDAE.

By PAUL D. HARWOOD.

From the Department of Biology, Rice Institute, Houston, Texas.

(With 1 Figure in the Text.)

Part I.

The examination of a water moccasin, *Agkistrodon piscivorus*, caught near Houston, Texas, on May 4th, 1932, revealed five species of parasites, four of which have not been previously reported from this host.

Two species of nematodes were present, including two specimens of *Kalicephalus agkistrodontis* Harwood 1932 found in the stomach, and a specimen of *Capillaria* found in the intestine. The latter form closely resembles *C. chelydrae* Harwood, in the character of the oesophagus, but because of its larger size and structural differences we consider it distinct; as only one female was found we shall reserve the description.

The only trematode encountered was *Renifer kansensis* Crow 1913, previously reported from the copperhead, *Agkistrodontis mokasen*, and the ground rattler, *Sistrurus miliaris*. In the present case five specimens were found in the mouth and lungs.

Two species of cestodes, both belonging to the *Ophiotaenia* group, were found in the small intestine. *Proteocephalus* (*Ophiotaenia*) *marenzelleri* (Barrois, 1889) was represented by ten large specimens. The descriptions of this parasite given by Schwarz (1908), Beddard (1913 *a*), and Woodland (1925 *c*) are for the most part adequate; but as there has been some disagreement on the condition of the inner longitudinal layer of muscles it is, perhaps, best to state that in my material the inner longitudinal muscle fibres were either absent or represented by only a few fibres which were not collected into bundles. It is interesting to note that the dorso-ventral fibres were both numerous and large.

Proteocephalus (Ophiotaenia) agkistrodontis n.sp.

The remaining parasite is also a member of the *Ophiotaenia* group, and as it seems to be new the name *Proteocephalus* (*Ophiotaenia*) *agkistrodontis* n.sp. is proposed for it. Fourteen specimens of this tapeworm were present, but only a few of them possessed mature segments. They were easily separated from *P.* (*O.*) *marenzelleri* because of their smaller slenderer form.

The scolex is large and sharply set off from the neck. It is 0·55–0·65 mm. long (from apex to caudal margin of suckers) by 0·85–0·95 mm. wide. The usual four suckers are directed outwards at right angles to each other and are 0·25–0·3 mm. in diameter. In whole mounts no trace of a fifth sucker or rostellum could be detected, but in the sections a few nuclei were noticed collected near the apex of the scolex. However, these nuclei could not be distinguished in any way from those of the sub-cuticular cells, and we believe that they were due merely to a cuticular fold. The musculature of the scolex and, indeed, of the whole tapeworm is very weak. There is a narrow band of muscle fibres along the edge of the suckers. A few weak dorso-ventral fibres as well as a poorly developed muscle star seem to be present. The eight bands of muscle fibres running from the suckers into the neck could not be located.

The neck is very long; in one specimen faint segmentation lines could be seen 5 mm. caudally to the scolex, but in other specimens the first signs of segmentation were the developing internal organs which do not appear until several centimetres behind the scolex. The neck is about 0·45 mm. wide at its narrowest point, and therefore only about half as wide as the scolex, but it is narrowest some distance behind the scolex, *i.e.* there is no sudden constriction. The first segments are 0·22 × 0·52 mm., and at this point only the outlines of the proglottids can be seen. When the first sign of the genital organs appears the segments measure 0·45 × 0·84 mm. This point is about 30 mm. from the head, and already elongation of the proglottids has commenced. Mature segments do not appear until well past the middle of the strobila. These first mature segments measure about 1·5 × 0·75 mm. The width continues to increase until a maximum of 0·9 mm. is reached, but by the time the last mature segment is reached the width has begun to diminish. These segments measure 2·6 × 0·8 mm. As the segments ripen elongation continues until the fully ripened segments may be five times as long as broad.

The details of the excretory system could not be worked out, due to the general loose condition of the parenchyma. In the scolex the tubes were well marked, and the usual ring of vessels is formed at the level of the suckers. The ducts leaving this ring seem to be single on each side in some sections, but double or even triple in others. As the scolex passes over into the neck the excretory ducts lose their distinctive staining reactions, and it is impossible to distinguish them from the spaces in the parenchyma. It is thought that within the strobila they form a network of irregularly branching tubes.

The muscular system is very weak. There is a weak sub-cuticular layer present, but no trace could be found of an inner longitudinal or circular layer. A few longitudinal fibres are present near the lateral margin, but their distribution was irregular, and they were not collected into bundles.

The genital pore lies between the caudal limits of the first fifth and the first fourth of the margin of the proglottid. There is a distinct genital papilla present and a small genital sinus. When fully protruded the cirrus may reach a length of 0·4 mm. It is slender, slightly swollen at the base and unarmed.

The ejaculatory duct is much coiled within the pouch even when the cirrus is protruded. The cirrus pouch is 0·23–0·27 × 0·09–0·111 mm. The coils of the vas deferens are rather loose, but extensive. They are most conspicuous in the ripe segments where they extend beyond the median line. The testes number from 90 to 110 per segment, and about 45 per cent. of the total number lie on the poral side of the segment. They are variable in size, the larger ones measuring about 0·075 mm. in diameter, the smaller about 0·035 mm., the average being about 0·06 mm. Their distribution is not quite typical of the *Ophiotaenia* group, as they may lie in the median line in that region of the proglottid which lies cephalic to the genital pore.

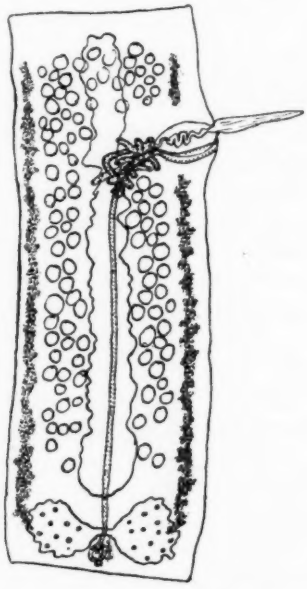


Fig. 1. Mature segment of *Proteocephalus* (*Ophiotaenia*) *agkistrodontis*.

The vagina may open either cephalic, caudal, or dorsal to the opening of the cirrus sac. As it passes mediad it may or may not pass over the cirrus sac and the coils of the vas deferens. Once it attains the mid-line it runs straight caudally without any coils. There is an enlargement of the vagina near the genital pore, and another, the so-called seminal vesicle, just cephalic to the ovarian bridge. The posterior vagina, unlike the anterior vagina, is much coiled within the inter-ovarian space. It passes over the bridge of the ovary and unites with the oviduct near the caudal margin of the inter-ovarian space. The usual organs are present in this space. The oöcapt is somewhat weakly developed, and a small but sharply defined shell gland is present. The ovary has two broad wings extending nearly to the vitellaria. Each lobe

is fairly solid and without blind pouches. The uterus is a central tube with much reduced pouches. There are from 25 to 30 uterine pouches on each side. There are no uterine pores present in the ordinary sense of the word, but the uterus splits open for the full length of its ventral surface, thus spilling its contents. Beddard (1913 *b*) used this character as the sole basis for founding the genus *Solanotaenia*, but as the same character is known among other proteocephalids of very different organisation, the genus cannot be accepted. The vitellaria are lateral and extend nearly the full length of the proglottid. The bands are usually a little wider and a little more dense near their caudal ends.

Habitat: Intestine of *Agkistrodon piscivorus*.

Locality: Houston, Texas.

Type specimen: U.S. National Museum.

It has not been possible to prepare a key to those species of the Proteocephalidae parasitising snakes, because only incomplete descriptions of a few of the species are available. The majority of the species may be separated from *Proteocephalus* (*Ophiotaenia*) *agkistrodontis* n.sp. by the position of the genital pore. This structure in *P.* (*O.*) *agkistrodontis* lies far forward, near the cephalic margin of the proglottid, while in more than half of this group of tapeworms it is at or near the middle of the margin, including both species of *Proteocephalus*, *P.* (*O.*) *marenzelleri* and *P.* (*O.*) *grandis*, which have been previously reported from the water moccasin. Of the remaining species two, *P.* (*O.*) *paraguayensis* and *P.* (*O.*) *san-bernardinensis*, may be easily separated by the characters of the muscular systems. In both species the inner longitudinal layer of muscle fibres is present, while in the former species the uterine pouches and in the latter species the vitellaria are cortical in position. *P.* (*O.*) *paraguayensis*, moreover, is distinguishable by its much greater number of testes, 260–310. *P.* (*O.*) *san-bernardinensis* has a very small scolex and suckers. This organ, only 0·25 mm. in diameter, bears suckers only 0·08–0·09 mm. in diameter, while in *P.* (*O.*) *agkistrodontis* all such measurements are about three times larger. *P.* (*O.*) *nattereri* also possesses a small scolex, the measurements being 0·24–0·25 mm. for the diameter of the scolex and 0·12–0·15 mm. for the diameter of the suckers. *P.* (*O.*) *flava*, *P.* (*O.*) *hyalina*, and *P.* (*O.*) *macrobothrium* are all three in many respects similar to the present species, but all three may be distinguished from it by their lesser number of testes per segment.

PART II. THE CHARACTERS OF THE PROTEOCEPHALIDAE AND THE MONTICELLIIDAE.

La Rue's (1914) monograph of the Proteocephalidae first placed that family in some degree of order, and his paper still remains the starting-point for any discussion on this subject. Since 1914 many departures from La Rue's system have been suggested until at the present time the classification of the family is again in a very confused state. Apparently the chief cause for the

confusion is that some of the characters which have been used to split the family run counter to others which have been used as a basis for generic distinctions. A comparison of the general anatomy, the host relationships, and the life histories, if known, of any group of animals should make it possible to decide which characters exhibited by those animals are fundamental, and which are trivial. This is what I propose to do for the characters of these two families.

La Rue (1911) first assigned family rank to those species of Proteocephalidae in which the inner longitudinal muscle sheath lay internal to the internal organs. He called his new family Monticelliidae because the only genus included in it at that time was named *Monticellia*. La Rue thereby assumed that the character upon which the genus *Monticellia* is based is more fundamental than the other characters which he and other workers had used to divide the Proteocephalidae.

Subsequently other forms have been discovered in which some but not all of the internal organs are situated external to the inner muscle layer. These worms were placed in separate genera by Woodland (1925 *a*, *b*) and others. Woodland, although accepting this character and its modifications as more fundamental than the others, did not allow the group family rank. Since other characters failed to run parallel to this one Woodland refused to recognise genera based on these other characters and limited the valid genera to *Proteocephalus* Weinland 1858, *Monticellia* La Rue 1911, *Marsypocephalus* Wedl 1861, and *Rudolphiella* Fuhrman 1916 (later declared to be a synonym of *Ephedrocephalus*).

Previous authors have apparently looked upon the relative positions of the muscle sheath and the internal organs as involving a shift of the latter from what is styled the medullary parenchyma to the cortical parenchyma. Any character based on such a shift of the internal organs would probably be fundamental in nature. But while I was working on this problem it was pointed out to me by Dr A. C. Chandler, of this Institution, that it is as easy to regard the internal organs as stationary and the muscle layer as the mobile organ. As the muscular system is known to be an extremely variable system in other groups of animals the fundamental nature of this character at once becomes questionable.

The worms which have been assigned to the family Monticelliidae are all parasites of siluroid fishes. They are all in general morphological agreement except that a few of them show a corolla-like folding of the cuticula just caudal to the suckers. This character has been the basis for the founding of *Corallobothrium* and other genera, but because it does not show any correlation with the monticelliid character Woodland (1925 *b*) refuses to recognise the genera based upon it. For the same reason, Fuhrman and Baer (1925) and Meggitt (1927) distribute those species showing this character to two families and several genera. Yet the corolla type of scolex is definitely limited to the parasites of siluroid fishes; it is easily recognised and distinctive. Why then

the tacit assumption that it is any less fundamental in nature than the monticelliid character?

If we consider these two characters from the standpoint of their value to the organism we are further puzzled by the attitude which various authors have taken toward them. It is difficult to imagine what possible function an internal position of the inner muscle sheath could serve in the case of these tapeworms; therefore, this character, unless linked to some valuable character, is not capable of becoming fixed by natural selection and its inheritance will be of the most erratic nature. In other words having once arisen it is very apt to be left free to appear or vanish according to the capricious dictates of chance. On the other hand the *Corallobothrium* type of scolex may and probably does serve the useful purpose of fastening the worm more firmly to the intestine of the host, and therefore, having once arisen, it is more likely to be preserved.

When we examine those Proteocephalidae which are not parasitic in catfishes we find ample evidence that characters involving the muscular system may be and, indeed, frequently are variable. In a number of these worms there is no inner muscular sheath, at least in the greater part of the strobila, and the distinction between medulla and cortex is entirely lost. These forms include *Proteocephalus tigrinus* Woodland, a number of species of the *Acanthotaenia* group, and *Proteocephalus* (*Ophiotaenia*) *agkistrodontis* n.sp. These tapeworms present such a diverse appearance that it is impossible to include them in a single group defined by such a character. *Ophiotaenia flava* Rudin 1917, like the above species, has lost all distinction between the cortex and medulla, but this species differs from the above-mentioned forms in that numerous longitudinal muscle fibres are present, but they are more or less indiscriminately scattered in the parenchyma. Of special interest in this connection is *Proteocephalus* (*Ophiotaenia*) *marenzelleri* (Barrois). Schwarz (1908) described the inner longitudinal muscle layer of this parasite as being weak. Beddard (1913 *a*) stated that such a layer could not be found in the greater part of the strobila in his material. Woodland (1925 *c*) described an inner longitudinal muscle layer for this species, and claimed that it was more conspicuous than in *Proteocephalus viperis* (= *Solanotaenia viperis* Beddard). Beddard (1913 *b*) described and figured an inner muscle sheath for *Solanotaenia viperis*. In some sections of my material no sheath of inner longitudinal muscle fibres could be located, while in others a few small fibres were present. However, these fibres were not collected into bundles, nor could they be interpreted as being as strong as those figured by Beddard for *Proteocephalus viperis*. It is obvious, therefore, that the inner longitudinal muscular layer may be extremely variable, even in a single species, and great caution must be exercised in assigning generic value to any variations of it.

Previous writers have been more or less cognizant of the above-mentioned variations, but they have either ignored them, or been unaware that in a number of Proteocephalidae not parasitic in siluroid fishes the inner muscle sheath is internal to certain of the internal organs. As two of these cases are

described fully in Rudin's (1917) important paper, it is difficult to imagine how such an oversight occurred. The vitellaria are external to the muscle sheath in *Acanthotaenia gallardi* Johnston 1911, *Ophiotaenia longmanni* Johnston 1916, *Ophiotaenia elapsoideae* Sandground 1928, and *Ophiotaenia sanbernardinensis* Rudin 1917, yet no one has suggested that these worms are related to the Monticelliidae. To be sure no genus of the family has yet been established with only the vitellaria in the cortex, yet the only difference which Woodland (1925 *b*) recognises between *Ephedrocephalus* and *Marsypocephalus* lies in the relative positions of the vitellaria and the muscle sheath. Therefore, if we accept these principles it is necessary to erect a new genus in the *Monticellia* group for them. But these four tapeworms are all parasites of snakes and in them the testes are divided into two lateral fields. Furthermore, *Acanthotaenia gallardi* possesses a thick coating of cuticular spinelets and a cone-shaped rostellum. It, therefore, is not related to the other three species. To include them all in one genus and place that genus in close relationship to *Monticellia* is to the last degree untenable. In *Ophiotaenia paraguayensis* Rudin 1917, the uterine stem lies between the fibres of the inner longitudinal muscle sheath, and the uterine pouches are entirely external thereto. The case here is similar to the above in every respect except the organ concerned, and it would, indeed, seem foolhardy to erect a new genus on the basis of this relation between the uterus and the muscular sheath.

Many of the existing descriptions make no mention of the relative positions of the internal organs and the inner muscle sheath. It is almost trite to remark, therefore, that when the present known forms are completely described it is to be expected that many more isolated variations of this character will be found, not only among the members of the *Ophiotaenia* group, but also among the members of *Proteocephalus* proper.

Since it is impossible to determine to which family several of these tapeworms belong, and since the *Monticellia* character does not show any correlation with either the *Corallobothrium* or the *Acanthotaenia* or the *Ophiotaenia* characters, and since the muscular system may be variable in its development even in a single species, I have concluded that the relative position of the internal organs and the inner longitudinal muscle sheath is a character of no more than specific importance.

Having discarded this as a generic character it is now necessary to review briefly the Proteocephalidae to see which genera may be preserved. But first it is necessary to give some further consideration to what constitutes a generic character.

For the greater part of the Proteocephalidae it is impossible to find clear-cut generic characters. I have assigned generic value to those characters which seem to group together a number of apparently closely related forms, judged from the standpoint of general anatomical resemblance and host relationship. Admittedly this is a somewhat arbitrary procedure in itself, and not entirely satisfactory. However, the variation of the characters necessitates such a

procedure in order to subdivide this large and heterogeneous group of cestodes.

As the genera *Monticellia* La Rue 1911, *Marsypocephalus* Wedl 1862, *Ephedrocephalus* Diesing 1860 (= *Rudolphiella* Fuhrman 1916), *Loennbergia* Fuhrman and Baer 1925, and *Geozeella* Fuhrman 1916 are all based on the relative positions of the inner muscle layer, they must in my opinion be discarded. The genera *Ophidotaenia* Beddard 1913, *Solanotaenia* Beddard 1913, and *Batrachotaenia* Rudin 1917 are too universally accepted as synonyms of *Ophiotaenia* La Rue 1911 to require further comment here. The genus *Choanoscolex* La Rue 1911 is likewise generally considered to be a synonym of *Corallobothrium*. Of the remaining generic names *Proteocephalus* being the oldest available for this group of animals must stand. *Lintoniella* Woodland 1927, *Corallobothrium* Fritsch 1886, and *Gangesia* Woodland 1925 are so well characterised by their peculiar features that they may be accepted without comment.

Most recent authors have refused to recognise the genus *Acanthotaenia*, but Baylis (1929) is of a different opinion. He writes, "The fact, however, remains that there is a series of forms, chiefly parasitic in species of *Varanus*, having the same character in common, viz. a covering of minute spines extending all over the scolex and usually over the whole or at least the anterior part of the strobila, and it is convenient to speak of them as '*Acanthotaenia*.'" The objections put forth against the validity of this genus by Woodland (1925 *b*) and accepted by others are that the minute spines are also present in certain species of cestodes usually referred to the genus *Gangesia*, and, furthermore, in *Proteocephalus osculatus*. The last-named worm is so poorly described that its systematic position must remain uncertain for the time being. Judging from present descriptions the scolex has a conical rostellum-like structure, which is surmounted by a fifth sucker. This fifth sucker bears a number of minute spines. It seems likely that *P. osculatus* may be considered to be an intermediate form linking both *Acanthotaenia* and *Gangesia* to *Proteocephalus*, and when better known it may be necessary to transfer it to one of the above genera. However, the spines are so small and so limited in distribution that this form may be easily separated from both of those genera, and therefore it seems best for *P. osculatus* to remain in the genus *Proteocephalus*. There is good reason to believe that *Gangesia* and *Acanthotaenia* are closely related, and that their spinelets may have been inherited from a common ancestor, but as the former possesses an armed rostellum they can readily be separated. Therefore, the presence of cuticular spinelets in *Gangesia* need cause no confusion, and it seems preferable to me to recognise the generic rank of *Acanthotaenia*.

In *Crepidobothrium* the caudal margin of the suckers is interrupted and reentrant into the cavity of the sucker. Nybelin (1917) has objected to this as a generic character. Nybelin's paper is among the three or four on the Proteocephalidae which are not available to me, so I cannot form a first-hand opinion

as to the nature of his argument, but judging from the comments which various authors have made on the subject, he seems merely to have considered the character as too trivial a basis for a genus. So far as I am aware this type of sucker does not occur elsewhere in the family, and therefore I see no reason why it should not be recognised.

The case of *Ophiotaenia* is much the same as that for *Acanthotaenia* except that it is much weaker. The *Ophiotaenia* group consists of a large number of cestodes which are almost certainly monophyletic in origin. The only workable distinction between the *Ophiotaenia* group and the rest of the genus *Proteocephalus* is the division of the testes into two fields. The appearance of the same character in *Gangesia* and *Acanthotaenia* need not cause any consternation, as it may have been inherited from a common ancestor, and those two genera are sufficiently well marked by their own peculiar characters. But in a number of *Ophiotaenia* species, particularly in those which are parasites of Amphibia, the division is incomplete, while in a number of the *Proteocephalus* species, particularly those from siluroid fishes, there is a more or less distinct division of the testicular field. So perfect is the gradation here that any division line must be so finely drawn that it will inevitably lead to considerable confusion. Yet so large are both groups that the preservation of some distinction is certainly desirable. Accordingly, I have assigned sub-generic rank to *Ophiotaenia* and have more or less arbitrarily disposed of the intermediate forms.

In the following paragraphs the genera of the Proteocephalidae are defined, and those new combinations which are necessitated by the suppression of *Monticellia* and related genera are listed in their proper places.

The genera of the Proteocephalidae.

Genus *Lintoniella* Woodland 1927.

With characters of the family: Scolex with four normal suckers, no hooks, no spines, no rostellum and no fold of tissue caudal to the suckers. *The vitellaria are concentric, i.e. they form a continuous sheet around the genital organs next within the longitudinal muscle layer.* Uterus normal, testes in one field. Parasites of Elasmobranchs.

Type species: *Lintoniella adherens* (Linton 1923).

Genus *Proteocephalus* Weinland 1858.

With characters of the family: Scolex with four normal suckers, spines absent, fifth sucker or a rostellum-like structure may be present, no hooks, and no fold of tissue just behind the four suckers. Vitellaria lateral, testes in one field or in two. Parasites of fishes, amphibians, and reptiles. Two subgenera.

Type species: *Proteocephalus filicollis* (Rudolphi).

Sub-genus *Proteocephalus* Weinland 1858.

With the characters of the genus: testes in one field (the developing uterus may push the testes in the midfield laterad). Parasites of fresh-water fishes.

Type species: *Proteocephalus filicollis* (Rudolphi 1802).

New combinations:

P. (*P.*) *coryphicephala* (Monticelli 1891) new comb. (= *Tetracotylus coryphicephalus* Monticelli 1891).

P. (*P.*) *heterobranchus* (Woodland 1925*a*) new comb. (= *Marsypocephalus heterobranchus* Woodland 1925).

P. (*P.*) *tanganyikae* (Fuhrman and Baer 1925) new comb. (= *Loennbergia tanganyikae* Fuhrman and Baer 1925).

P. (*P.*) *rectangularis* (Wedl. 1862) new comb. (= *Marsypocephalus rectangularis* Wedl 1862).

Sub-genus *Ophiotaenia* La Rue 1911.

With the characters of the genus: The testes are divided into two fields leaving a clear median field. Parasites of amphibians and reptiles.

Type species: *Proteocephalus* (*Ophiotaenia*) *perspicua* La Rue 1911.

Genus *Crepidobothrium* Monticelli 1899.

With characters of the family: Head with four suckers *in which the caudal margin is interrupted and re-entrant.* No hooks, no spines, no rostellum and no fold of tissue caudal to the suckers. Testes in two fields, vitellaria normal. Parasites of snakes.

Type species: *Crepidobothrium gerrardii* (Baird 1860).

Genus *Acanthotaenia* v. Linstow 1903.

With characters of the family: Scolex with four suckers, *cone-shaped rostellum present, but it lacks hooks, numerous spinelets present on scolex and often on strobila.* No fold of tissue on scolex. Testes in one or two fields, vitellaria lateral. Parasites of reptiles, mainly *Varanus* spp.

Type species: *Acanthotaenia shipleyi* v. Linstow 1903.

Genus *Gangesia* Woodland 1925.

With characters of the family: Scolex with four normal suckers, no fifth sucker, no fold of tissue caudal to the suckers, spines may be present, *a rostellum armed with hooks is present.* Testes in one or two fields, vitellaria lateral. Parasites of siluroid fishes.

Type species: *Gangesia bengalensis* (Southwell 1913).

Genus *Corallobothrium* Fritsch 1886.

With the characters of the family: Scolex with four normal suckers, no rostellum, no hooks, no spines, *a fold of tissue lies caudal to the suckers and may overlap them.* Testes in one field, vitellaria lateral, or slightly dorsal. Parasites of siluroid fishes.

Type species: *Corallobothrium solidum* Fritsch 1886.

New combination:

C. siluri (Fuhrman 1916) new comb. (= *Goezeella siluri* Fuhrman 1916).

Part III. The phylogeny of the Proteocephalidae.

It is always of interest to speculate on the phylogenetic development of any group of animals, even though we are limited to a consideration of the final products of that development. Among the cestodes there are no means of bridging the gaps between living forms, as fossils of extinct forms are not known, and because of the nature of these animals, as well as the character of their habitat, it seems very unlikely that we shall ever possess much knowledge of the extinct forms. Thus the nature of the material compels our speculations to be highly hypothetical in nature, but none the less interesting.

La Rue (1914) and Rudin (1917) have both considered this subject in a very conservative manner, but in view of our present more extensive knowledge of these parasites it has seemed to me possible to carry the speculations much further.

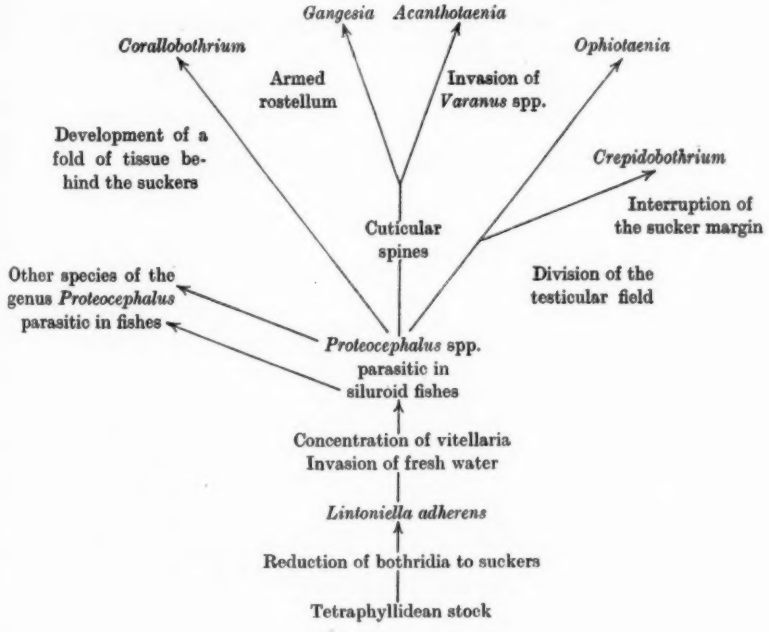
The Proteocephalidae have been most often referred to the Tetraphyllidea, to which order they are undoubtedly most closely related. The Tetraphyllidea are mostly marine, while the only marine proteocephalid is *Lintoniella adherens*, a parasite of sharks. The vitellaria of this worm are concentric in arrangement, and because of this character, and also because of its habitat I consider *Lintoniella* to be the most primitive of the Proteocephalidae.

La Rue (1914) suggested that *Proteocephalus singularis*, a parasite of ganoid fishes, might be the most primitive of the fresh-water Proteocephalidae, but the only possible primitive feature which this form possesses lies in the shape of its suckers. *Lintoniella* on the other hand has a normal scolex, but is peculiar in the distribution of the vitellaria. If we search among the *Proteocephalus* species for forms with peculiar vitellaria we find them among the parasites of siluroid fishes. In *P. coryphicephalus* the vitellaria are arranged in broad, slightly dorsal bands, instead of the usual compact lateral rows.

But the siluroid fishes were not differentiated from the other fishes until the Cretaceous Period, which is comparatively late in geological times. Therefore, if they are to be considered responsible for the introduction of the Proteocephalidae into fresh water certain other conditions must be fulfilled. The siluroid fishes must have a wide distribution in both fresh and salt water, and there should exist evidence that the Proteocephalidae have spread independently from the siluroid fishes to such other vertebrate groups as the remaining fresh-water fishes, the lizards and the snakes. That the first condition need give us no concern is too generally known to require further comment, but the second needs considerable explanation. The species of the sub-genus *Proteocephalus* form such a compact interrelated group that they could easily have been derived from almost any member of the sub-genus. The genus *Corallobothrium* is entirely confined to catfishes, and further such intermediate forms

as *Proteocephalus beauchampi* and *P. tanganyikae* are parasitic in siluroid fishes. Therefore, we may assume that *Corallobothrium* arose among the siluroid fishes, and as some of the members of this genus possess vitellaria arranged in broad bands we may also assume that their origin came very soon after the invasion of the fresh-water habitat. *Gangesia* is another genus confined to siluroid fishes, and an intermediate form is known under the name of *Proteocephalus osculatus*, as a parasite of German siluroids. Members of the genus *Acanthotaenia* are parasites of lizards and rarely of snakes, but the presence of cuticular spinelets and of a conical rostellum-like structure places this genus in close relationship with *Gangesia*. The *Ophiotaenia* group is but poorly defined by its character of the two field distribution of testes. It might equally well have arisen from several places in the sub-genus *Proteocephalus*, but no origin seems more likely from the morphological view-point than the parasites of the siluroid fishes in which partial division of the testicular field is often present. Indeed, as both *Gangesia* and *Acanthotaenia* often present a more or less divided testicular field I should place the origin of *Ophiotaenia* close to those two genera. *Crepidobothrium* of course arose from the *Ophiotaenia* stock.

The following chart summarises the above discussion. Future research will doubtless introduce considerable modifications, and it seems most likely that evidence which will either support this hypothesis or render it untenable will be found among the parasites of the marine siluroid fishes.



REFERENCES.

BAYLIS, H. A. (1929). On a larval form of *Acanthotaenia*. *Ann. Mag. Nat. Hist.* **4**, 224.

BEDDARD, F. E. (1913 *a*). On some species of *Ichthyotaenia* and *Ophidotaenia* from Ophidia. *Proc. Zool. Soc. London*, **1913**, 153.

—— (1913 *b*). On a new genus of *Ichthyotaeniids*. *Ibid.* 243.

FUHRMAN, O. and BAER, J. G. (1925). Zoological results of the third Tanganyika expedition conducted by Dr W. A. Cunnington 1904–1905. Report on Cestoda. *Ibid.* **1925**, 79.

LA RUE, G. R. (1911). A revision of the cestode family Proteocephalidae. *Zool. Anz.* **38**, 473.

—— (1914). A revision of the cestode family Proteocephalidae. *Illinois Biol. Monographs*, **1**, 1.

MEGGITT, F. J. (1927). Remarks on the cestode families Monticelliidae and Ichthyotaeniidae. *Ann. Trop. Med. and Parasitol.* **21**, 69.

NYBELIN, O. (1917). Results of Dr E. Mjöberg's Swedish scientific expedition to Australia, 1910–1913. XIV. Australische Cestoden. *K. Svenska Vet.-Ak. Handl. Stockholm*, **25**, 1.

RUDIN, E. (1917). Die Ichthyotaenien der Reptilien. *Rev. Suisse Zool.* **25**, 179.

SCHWARZ, R. (1908). Die Ichthyotaenien der Reptilien und Beiträge zur Kenntnis der Bothriocephalen. *Inaug. Dissert. Univ. Basle*, 1.

WOODLAND, W. N. F. (1924). On a new *Bothriocephalus* and a new genus of Proteocephalidae from Indian freshwater fishes. *Parasitology*, **16**, 424.

—— (1925 *a*). On some remarkable new *Monticellia*-like and other cestodes from Sudanese siluroids. *Quart. J. Micr. Sci.* **69**, 703.

—— (1925 *b*). On three new proteocephalids (Cestoda) and a revision of the genera of the family. *Parasitology*, **17**, 370.

—— (1925 *c*). On *Proteocephalus marenzelleri*, *P. naiae* and *P. viperis*. *Ann. Trop. Med. and Parasitol.* **19**, 265.

—— (1927). A revised classification of the Tetraphyllidean Cestoda, with descriptions of some Phyllobothriidae from Plymouth. *Proc. Zool. Soc. London*, **1927**, 519.

(*MS. received for publication* 31. VIII. 1932.—Ed.)